($CDCl_3$) δ 0.84 (3 H, s), 1.76 (6 H, s), 2.68–3.40 (12 H, m), 6.64 (2 H, s), 6.72 (1 H, s), 7.62 (2 H, d, J = 2 Hz), 7.74 (2 H, d, J = 2 Hz), 7.86 (2 H, s). Anal. Calcd for $C_{33}H_{30}O_3Br_6$: C, 41.55; H, 3.17. Found: C, 41.21; H, 3.42.

Reaction of 12d with *N,N*-Dimethylaniline. After a solution of 25 mg (0.026 mmol) of **12d** and 0.5 mL of *N,N*-dimethylaniline in 4 mL of EtOAc was stirred at room temperature for 2 h, the solvent was evaporated in vacuo. The residue was washed with a small amount of EtOAc to give 23 mg (80%) of ***N*-benzyl-*N,N*-dimethyl-*N*-{2-oxo-2-[5′-{13′,21′,29′-tris(bromoacetyl)-8′,16′,24′,32′-tetramethyl[2.2.2.2]metacyclophano}]ethyl}ammonium bromide (20)** as a colorless powder, mp 129–134 °C dec: IR (KBr) ν C=O 1680 cm^{-1}. Anal. Calcd for $C_{52}H_{55}O_4NBr_4$: C, 57.96; H, 5.14; N, 1.30. Found: C, 58.19; H, 5.08; N, 1.31.

Registry No. **1**, 67691-33-2; **2a**, 119877-85-9; **2b**, 77180-45-1; **3**, 119877-86-0; **4**, 119877-87-1; **5**, 119877-88-2; **6**, 119877-89-3; **7**, 119877-90-6; **8**, 119877-91-7; **9**, 119877-92-8; **10**, 119877-93-9; **11a**, 108-24-7; **11b**, 75-36-5; **11c**, 22118-09-8; **11d**, 598-21-0; **11e**, 18255-47-5; **12a**, 119877-94-0; **12b**, 119878-09-0; **12c**, 119877-95-1; **12d**, 119877-96-2; **12e**, 119877-97-3; **13a**, 119877-98-4; **13b**, 119877-99-5; **13c**, 119878-00-1; **13d**, 119878-01-2; **13e**, 119878-02-3; **14**, 119878-03-4; **15**, 119878-04-5; **16**, 119878-05-6; **17**, 119878-06-7; **18**, 119878-07-8; **20**, 119878-08-9; *N,N*-dimethylaniline, 121-69-7.

Synthesis and Vesicle Formation of Identical- and Mixed-Chain Di-*n*-alkyl Phosphate Amphiphiles

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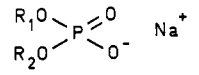
The synthesis of identical-chain and mixed-chain di-*n*-alkyl phosphate amphiphiles $(C_nH_{2n+1}O)(C_mH_{2m+1}O)PO_2^-Na^+$ (**1–13**) is described (n = 10, 12, 14, 16, m = 10, 12, 14, 16; n = 10, 12, 14, m = 18). Despite large differences in the hydrocarbon chains, these amphiphiles, when suspended in an aqueous solution, all form bilayer vesicles as revealed by electron microscopy. With 1,6-diphenylhexatriene as a hydrophobic fluorescent probe, fluorescence polarization values were determined over a range of temperatures. Phase-transition temperatures (T_m) for the transition from a gellike to a liquid-crystalline phase were derived, except for the vesicles formed from the most asymmetric phosphates (n = 10, 12, m = 18). The T_m values decrease with decreasing chain lengths and increasing asymmetry of the alkyl chains. The temperature dependence of the linewidth of the ^{31}P NMR resonance of the vesicles is briefly discussed.

Introduction

Important aspects of the chemistry of biological cell membranes can be successfully mimicked by using bilayer vesicles formed from naturally occurring or synthetic phospholipids.[1] Subsequent pioneering work by Kunitake[2] and others[3] has shown that simple, synthetic double-chain amphiphiles also aggregate to form vesicular assemblies with properties very similar to those of phospholipid vesicles. These developments provide challenging possibilities for investigating the effects of structural variations in the amphiphile on the structural and functional properties of the vesicle bilayers. Therefore, these vesicles offer attractive model systems for analyzing fundamental processes that may be of relevance to a detailed understanding of the properties of biological membranes. These processes include morphological changes,[4,5] lateral and flip-flop movement of amphiphiles in the bilayer,[6,7] osmotic activity,[8,9] and, as demonstrated recently, membrane fusion.[10,11] Herein, we describe the synthesis and phase behavior of a series of saturated di-*n*-alkyl phosphate amphiphiles in which the alkyl chains are equal in length (**1, 6, 10, 13**) or differ in carbon number (**2–5, 7–9, 11, 12**). We find that all amphiphiles readily form vesicles despite the obviously large differences in the total free energy of chain packing in the bilayer as a consequence of the large differences in lengths of both *n*-alkyl chains. The phase-transition temperatures (T_m) for the transition from the



1: $R_1 = R_2 = n\text{-}C_{10}H_{21}$
2: $R_1 = n\text{-}C_{10}H_{21}$; $R_2 = n\text{-}C_{12}H_{25}$
3: $R_1 = n\text{-}C_{10}H_{21}$; $R_2 = n\text{-}C_{14}H_{29}$
4: $R_1 = n\text{-}C_{10}H_{21}$; $R_2 = n\text{-}C_{16}H_{33}$
5: $R_1 = n\text{-}C_{10}H_{21}$; $R_2 = n\text{-}C_{18}H_{37}$
6: $R_1 = R_2 = n\text{-}C_{12}H_{25}$
7: $R_1 = n\text{-}C_{12}H_{25}$; $R_2 = n\text{-}C_{14}H_{29}$
8: $R_1 = n\text{-}C_{12}H_{25}$; $R_2 = n\text{-}C_{16}H_{33}$
9: $R_1 = n\text{-}C_{12}H_{25}$; $R_2 = n\text{-}C_{18}H_{37}$
10: $R_1 = R_2 = n\text{-}C_{14}H_{29}$
11: $R_1 = n\text{-}C_{14}H_{29}$, $R_2 = n\text{-}C_{16}H_{33}$
12: $R_1 = n\text{-}C_{14}H_{29}$; $R_2 = n\text{-}C_{18}H_{37}$
13: $R_1 = R_2 = n\text{-}C_{16}H_{33}$

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Table I. Synthesis of Di-*n*-alkyl Hydrogen Phosphates

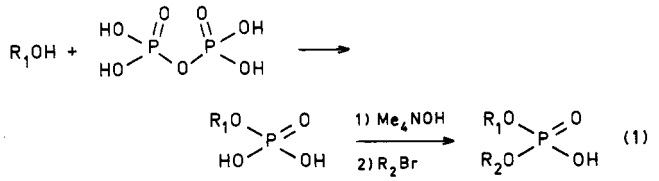
compd	R_1	R_2	yield,[a] %	mp, °C (solvent)	^{31}P NMR[b] δ, ppm
1a	n-$C_{10}H_{21}$	n-$C_{10}H_{21}$	81	44–46 (hexane)	1.25
2a	n-$C_{10}H_{21}$	n-$C_{12}H_{25}$	94	42–44 (hexane)	1.26
3a	n-$C_{10}H_{21}$	n-$C_{14}H_{29}$	80	49–52 (hexane)	1.32
4a	n-$C_{10}H_{21}$	n-$C_{16}H_{33}$	92	58–59 (hexane)	1.34
5a	n-$C_{10}H_{21}$	n-$C_{18}H_{37}$	83	61–62 (hexane)	1.33
6a	n-$C_{12}H_{25}$	n-$C_{12}H_{25}$	*c*	60–61 (ether)	1.36
7a	n-$C_{12}H_{25}$	n-$C_{14}H_{29}$	97	55–59 (hexane)	1.36
8a	n-$C_{12}H_{25}$	n-$C_{16}H_{33}$	94	60–62 (hexane)	1.33
9a	n-$C_{12}H_{25}$	n-$C_{18}H_{37}$	72	65–66 (hexane)	1.41
10a	n-$C_{14}H_{29}$	n-$C_{14}H_{29}$	90	69–70 (methanol)	1.36
11a	n-$C_{14}H_{29}$	n-$C_{16}H_{33}$	78	64–66 (hexane)	1.30
12a	n-$C_{14}H_{29}$	n-$C_{18}H_{37}$	52	68–69 (methanol)	1.27
13a	n-$C_{16}H_{33}$	n-$C_{16}H_{33}$	*c*	74–75 (CH_2Cl_2)	1.27

[a] Second alkylation step (eq 1). [b] 0.4 M solution in $CHCl_3$ at 30 °C. [c] Commercially available.

frozen gellike state to the fluid liquid crystalline state, as determined by using a fluorescence polarization technique, were found to respond to *n*-alkyl chain length variation. Both the total number of carbon atoms in the chains and the difference in length between the chains are major parameters in determining T_m and the cooperativity in the phase transition.[12] The temperature dependences of the ^{31}P NMR resonances illustrate complex differences in bilayer packing and headgroup hydration for vesicles formed from **1–13**.

Results and Discussion

Synthesis. Although mixed diesters of phosphoric acid have been described in the literature,[13-15] the synthetic procedures are almost exclusively focused on short-chain diesters. We synthesized **1a–12a** via the alkyl dihydrogen phosphate according to the procedure (eq 1) reported by



(1)

Bauman.[16] The yields of the second alkylation step are satisfactory (Table I). No tri-*n*-alkyl phosphates were detected in the reaction mixtures. Small quantities of the alkyl dihydrogen phosphate could be removed by crystallization.

Vesicle Formation. Vesicles can be prepared by a variety of standard procedures.[1] Using the ethanol injection method (see the Experimental Section), we find that the sodium salts derived from **1–13** all form vesicles as evidenced by electron microscopy (vide infra). Thus, formation of the vesicle bilayer is not precluded by the sometimes large asymmetry in the hydrocarbon chain lengths in the amphiphile (e.g. difference of eight methylene groups in **5**). In all cases the vesicles were prepared at temperatures above T_m (cf. Table II) and were found to be stable for at least several hours. For electron microscopy, ^{31}P NMR spectroscopy, and fluorescence polarization measurements freshly prepared vesicle solutions were used. When the vesicle preparations are cooled below T_m, crystallization tends to occur, particularly for **1** and **2**.

Table II. Fluorescence Polarizations (P) and Phase-Transition Temperatures (T_m) of Vesicles Formed from 1–13

amphiphile[a]	P[b]	T_m, °C
1 (55)	0.34 (2), 0.31 (8), 0.17 (50)	8
2 (52)	0.36 (4), 0.25 (13), 0.08 (37)	13
3 (60)	0.36 (4), 0.24 (24), 0.10 (56)	24
4 (60)	0.34 (8), 0.19 (32), 0.10 (57)	[28][c]
5 (61)	0.33 (10), 0.26 (21), 0.08 (54)	*d*
6 (55)	0.39 (12), 0.29 (28), 0.10 (50)[e]	28
7 (61)	0.39 (4), 0.27 (32), 0.10 (52)	32
8 (61)	0.40 (7), 0.26 (39), 0.08 (59)	39
9 (66)	0.36 (4), 0.23 (35), 0.10 (66)	*c*
10 (70)	0.39 (17), 0.28 (48), 0.12 (62)	48
11 (62)	0.36 (10), 0.16 (48), 0.09 (62)	48
12 (70)	0.33 (6), 0.22 (38), 0.08 (68)	*c*
13 (70)	0.37 (25), 0.23 (66), 0.11 (79)	66

[a] The temperature (°C) at which the vesicles were prepared is given in parentheses. [b] Temperatures (°C) given in parentheses. [c] Weakly cooperative transition. [d] Noncooperative transition. [e] Data from ref 10b.

Electron Microscopy. Initially, the samples for electron microscopic investigation were prepared by placing them on positively charged carbon-coated Formvar grids, followed by staining with 1% (w/v) solutions of uranyl acetate. Under these conditions, aggregated vesicles were observed, the extent of aggregation decreasing with decreasing vesicle concentration. No aggregation at all occurred in the absence of uranyl acetate (**2**, **3**). Generally the diameter of the vesicles was less than 1000 Å. The use of uncharged grids led to aggregated vesicles for **1**, **7**, **8**, **11**, and **13**. No or very little aggregation was observed for vesicles formed from **2**, **4**, and **5**. Vesicles prepared from **10** and stained with uranyl acetate at 70 °C (e.g. above T_m) showed fusion to large vesicles with diameters as large as 10.000 Å (cf. Figure 7 in ref 10d). At low uranyl acetate concentration (0.01 and 0.1% w/v) only aggregation, but no fusion, was observed. Apparently, uranyl acetate induces aggregation, and above a certain threshold concentration, fusion of the vesicles. Cryo-electron and "thin-section"[17] electron microscopy indicated that the vesicles

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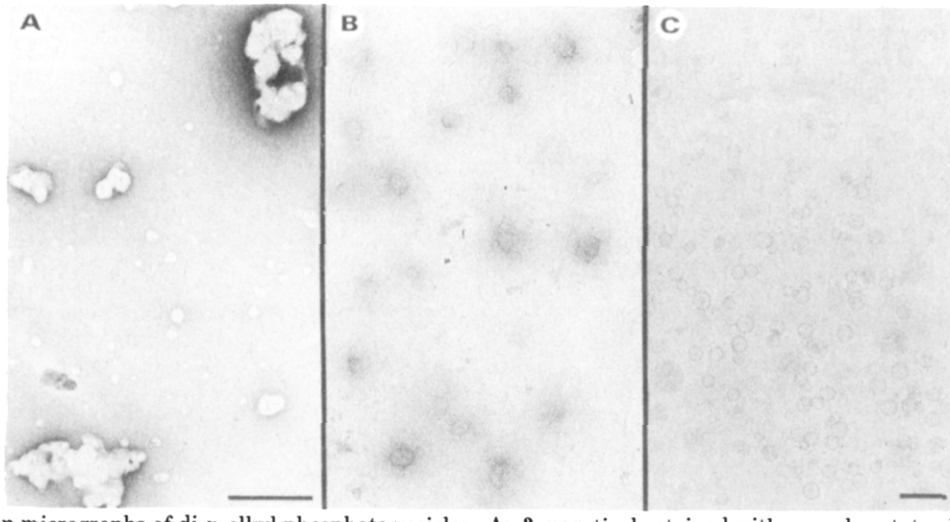

Figure 1. Electron micrographs of di-*n*-alkyl phosphate vesicles. A: **3**, negatively stained with uranyl acetate. B: **3**, in the absence of uranyl acetate. C: cryo-electron micrograph of vesicles formed from **2**. For A and B the marker line shown in A represents 5000 Å; for C, 1000 Å.

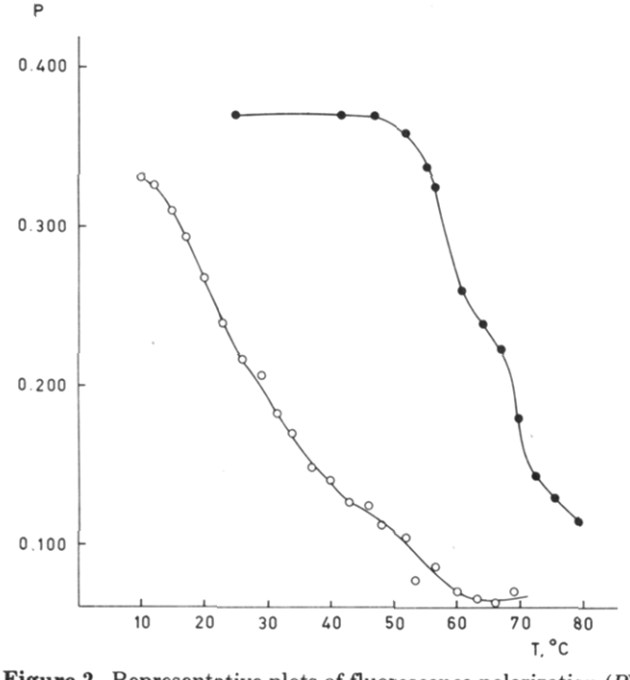

Figure 2. Representative plots of fluorescence polarization (P) vs T (°C) for **13** (●, cooperative transition) and **5** (○, noncooperative transition).

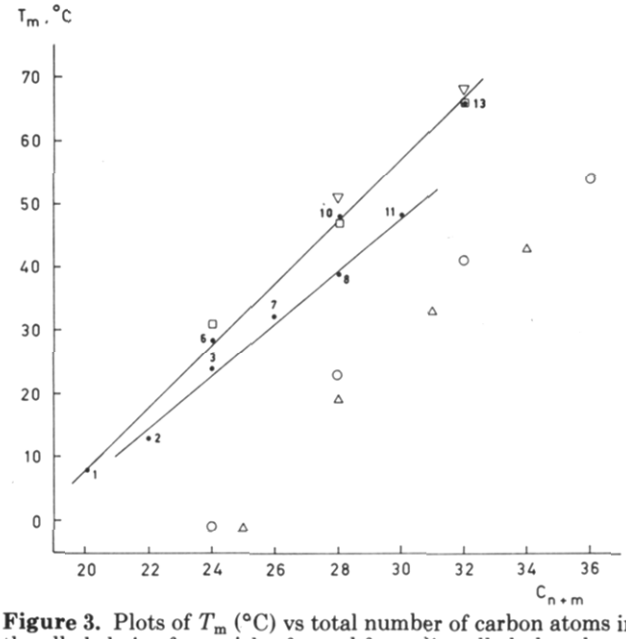

Figure 3. Plots of T_m (°C) vs total number of carbon atoms in the alkyl chains for vesicles formed from di-*n*-alkyl phosphates (●), phosphatidyl cholines, $n = m$ (○), phosphatidyl cholines, $n \neq m$ (△), phosphatidylethanolamines, $n = m$ (□), and phosphatidic acids, $n = m$ (▽).

have a unilamellar structure. Representative micrographs are shown in Figure 1.

Fluorescence Polarization. This technique is highly useful for probing the fluidity of the interior of the vesicle bilayer and for measuring the temperature (T_m) at which the bilayer undergoes a transition from a gellike to a liquid–crystalline state.[18] We have measured the fluorescence polarization (P) as a function of the temperature using 1,6-diphenylhexatriene (DPH), a hydrophobic fluorescent probe which intercalates between the alkyl chains in the core of the vesicle bilayer.[19] Fluorescence polarization values and phase-transition temperatures for vesicles formed from **1–13** are summarized in Table II. Emphasis is placed on the main phase transition, a more detailed analysis of the phase-transition behavior must await X-ray diffraction experiments.[20] We find that the overall changes in P values, upon going from the gellike to the liquid-crystalline state are quite comparable for the different vesicles. However, sharp phase-transition temperatures, indicating a highly cooperative main phase transition, are found for **6–8**, **10**, **11**, **13** (Figure 2), whereas the vesicles formed from **1–5** exhibit a more gradual transition. A weakly cooperative transition is found for **4**, **9**, and **12**, but particularly **5**, with a large difference in alkyl chain length, shows a smooth decrease of P with increasing temperature and a well-defined T_m cannot be identified (Figure 2). Plots of T_m vs the total number of carbon atoms (C_{n+m}) in R_1 and R_2 are shown in Figure 3. Two, slightly different, linear plots are obtained. For $n = m$, T_m increases 9.8 °C per two-carbon unit added to the alkyl chains, whereas in the case of $n \neq m$, a value of 8.4 °C is found. Interestingly, almost identical T_m values are

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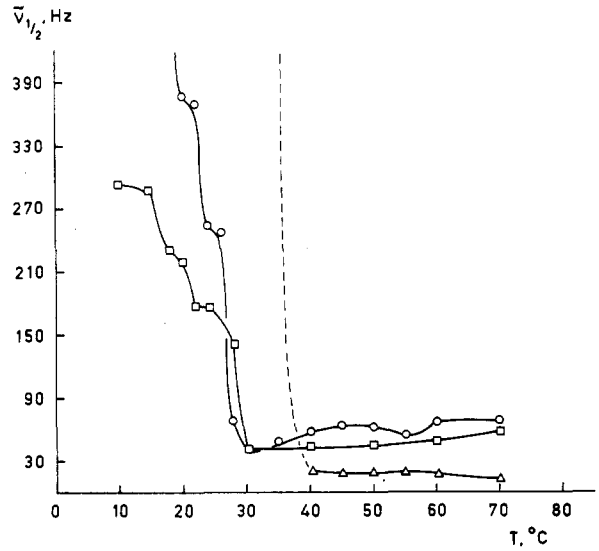

Figure 4. Plots of the linewidth of the ^{31}P NMR resonance vs temperature for vesicles formed from **7** (○), **8** (□), and **10** (△). Vesicles formed from **11–13** show essentially similar behavior.

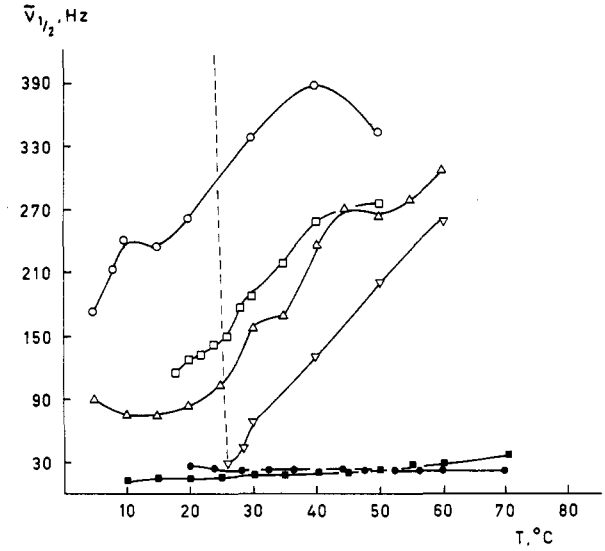

Figure 5. Plots of the linewidth of the ^{31}P NMR resonance vs temperature (°C) for vesicles formed from **1** (○), **2** (□), **3** (△), **6** (▽), **4** (●), and **5** (■). Vesicles formed from **9** show the same behavior as **4** and **5**.

found for the identical-chain phosphates (**1**, **6**, **10**, and **13**) and for identical-chain phosphatidylethanolamine (PE)[21] and phosphatidic acid (PA)[22] vesicles. The T_m values for phosphatidyl choline (PC)[21] vesicles are much lower at the same $n + m$ (Figure 3), but the chain-length dependence and the effect of differences in chain length ($n = m$ vs $n \neq m$) on T_m are comparable for the phosphates and the phospholipids. In the case of mixed-chain phosphates, the T_m values are lower for the same $n + m$ as compared with the symmetric phosphates and the cooperativity of the phase transition rapidly decreases with increasing difference between the lengths of the two alkyl chains. Apparently, at the same $n + m$, chain packing in the gel state is less effective if $n \neq m$. As discussed in some detail for mixed-chain phospholipid bilayers,[20] optimum van der Waals interaction between the hydrocarbon chains may be obtained by interdigitation of the chains across the center of the bilayer. Although voids in the chain packing can be avoided by this mechanism, it is found that increasing requirement for interdigitation is accompanied by lower T_m values and lower enthalpies for the phase transition.

^{31}P NMR Spectroscopy. Extensive work in the field of phospholipid vesicles[7,23] and vesicles formed from amphiphilic dialkyl phosphates[10d] has shown that ^{31}P NMR spectroscopy is a valuable tool in the study of structural aspects of the bilayer and, in particular, the headgroup region. We have measured peak intensities and line widths of the ^{31}P NMR resonances of vesicles formed from **1-13** over a temperature range around T_m. Three types of behavior were observed. Figure 4 shows plots of the linewidth vs temperature for the longer chain amphiphiles ($n + m$ = 26–32) with a difference in length of the alkyl chains of no more than four methylene units (**7**, **8**, **10–13**). At $T > T_m$ a relatively sharp and isotropic signal (linewidth ca. 45 Hz) is obtained, characteristic for normal bilayer packing in the liquid crystalline state.[10d,23c] Cooling of the vesicle solution to temperatures around T_m leads to an abrupt and large increase of the linewidth, indicative for partial freezing of the headgroup rotation on the ^{31}P NMR time scale when the bilayer undergoes a phase transition to the gel state. Quite different behavior is found for the shorter chain amphiphiles ($n + m$ = 20–24; **1–3**, **6**). Now the linewidth increases at $T > T_m$ (Figure 5). We suggest that this effect is caused by dehydration and clustering of the headgroups upon increasing the temperature. Clustering of the headgroups will be accompanied by bulging of the hydrocarbon chains and a concomitant loss of van der Waals contacts. This may explain why these effects only occur for the shorter alkyl chains in the amphiphiles. Generally we find for these vesicles a downfield shift of the ^{31}P NMR signal with increasing temperature, most likely due to a combination of several factors, including dehydration[10d,24] and increased counterion binding.[25] A stereoelectric effect[26] may also be involved since clustering may provide the necessary space in the headgroup region for the operation of this effect. Finally, the two amphiphiles with the highest asymmetry in the alkyl chains (**4**, **5**, and **9**) show narrow ^{31}P NMR resonances between 10 and 60 °C (Figure 5). Apparently, the change in order of the alkyl chains as indicated by the fluorescence polarization values (Table II) does not affect the dynamics of the polar headgroups, which may be rationalized by assuming that partial interdigitation[20] of the alkyl chains leads to a packing regime that offers more space to the phosphate moieties at the surface of the vesicle. In fact, for these highly asymmetric phosphates a "triple-chain" structure[27] of the gel phase may be envisioned, but more detailed structural investigation is required before definite conclusions can be drawn.

Experimental Section

Materials. Didodecyl hydrogen phosphate was obtained from Alpha Chemicals, dihexadecyl hydrogen phosphate and Hepes from Sigma, diphenylhexatriene (DPH) from Aldrich, and py-

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rophosphoric acid from Fluka. The long-chain alcohols and 1-bromoalkanes were purchased from Janssen.

Synthesis. The *n*-alkyl dihydrogen phosphates were prepared according to a literature procedure.[28] The di-*n*-alkyl hydrogen phosphates were synthesized by a procedure analogous to that given by Bauman[16] (see Table I) and purified by crystallization. The ^1H NMR spectra (solutions in $CDCl_3$ and TMS as an internal standard) were taken at 300 MHz. The spectral data are in line with the proposed structures, e.g. δ = 0.89 (CH_3, t), 1.26 ($(CH_2)_n$, chain), 1.68 (CH_2, m), 4.03 ppm (CH_2O, double t, $J_{^{31}P}$ = 6.7 Hz). A complete description is given for **10a** (vide infra). ^1H-decoupled ^{31}P NMR data were obtained with a spectrometer operating at 80.988 MHz and equipped with a temperature controller and deuterium lock. Samples were contained in 10-mm tubes. Chemical shifts (ppm) were determined relative to hexachlorocyclotriphosphazene (+19.9 ppm downfield from 85% H_3PO_4) in $CDCl_3$ as an external reference. The ^{31}P chemical shifts are concentration and temperature dependent (Table I).

Di-*n*-decyl hydrogen phosphate (1a): mp 44–46 °C (lit.[29] mp 33–34 °C).

***n*-Decyl *n*-dodecyl hydrogen phosphate (2a)**: mp 42–44 °C. Found: C, 64.54; H, 11.67; P, 7.64. $C_{22}H_{47}O_4P$ requires: C, 64.99; H, 11.65; P, 7.62. Sodium salt, found: C, 61.62; H, 10.74. $C_{22}H_{46}O_4PNa$ requires: C, 61.66; H, 10.82.

***n*-Decyl *n*-tetradecyl hydrogen phosphate (3a)**: mp 49–52 °C. Found: C, 66.44; H, 11.81; P, 7.54. $C_{24}H_{51}O_4P$ requires: C, 66.32; H, 11.83; P, 7.13.

***n*-Decyl *n*-hexadecyl hydrogen phosphate (4a)**: mp 58–59 °C (lit.[16] mp 57–58 °C).

***n*-Decyl *n*-octadecyl hydrogen phosphate (5a)**: mp 61–62 °C. Found: C, 68.32; H, 12.08; P, 6.29. $C_{28}H_{59}O_4P$ requires: C, 68.53; H, 12.12; P, 6.31.

Di-*n*-dodecyl hydrogen phosphate (6a): mp 60–61 °C (lit.[30] mp 59.1–60.2 °C).

***n*-Dodecyl *n*-tetradecyl hydrogen phosphate (7a)**: mp 55–59 °C. Found: C, 67.45; H, 11.95; P, 6.80. $C_{28}H_{55}O_4P$ requires: C, 67.49; H, 11.98; P, 6.69.

***n*-Dodecyl *n*-hexadecyl hydrogen phosphate (8a)**: mp 60–62 °C. Found: C, 68.12; H, 12.08; P, 6.41. $C_{28}H_{59}O_4P$ requires: C, 68.53; H, 12.12; P, 6.31.

***n*-Dodecyl *n*-octadecyl hydrogen phosphate (9a)**: mp 65–66 °C. Found: C, 69.35; H, 12.22; P, 6.02. $C_{30}H_{63}O_4P$ requires: C, 69.45; H, 12.24; P, 5.97.

Di-*n*-tetradecyl hydrogen phosphate (10a): mp 69–70 °C (lit.[30] mp 68–69 °C); ^1H NMR ($CDCl_3$) δ 0.88 (t, $J_{13,14}$ = 7.2 Hz, 6 H), 1.25 (m, 44 H), 1.67 (m, 4 H), 4.01 (dt, $J_{1,2}$ = 7.4 Hz, $J_{^{31}P}$ = 6.6 Hz), 8.5 (s, 1 H) ppm.

***n*-Tetradecyl *n*-hexadecyl hydrogen phosphate (11a)**: mp 64–66 °C. Found: C, 69.33; H, 12.31; P, 5.65. $C_{30}H_{63}O_4P$ requires: C, 69.45; H, 12.24; P, 5.97.

***n*-Tetradecyl *n*-octadecyl hydrogen phosphate (12a)**: mp 68–69 °C. Found: C, 70.04; H, 12.32; P, 5.65. $C_{32}H_{67}O_4P$ requires: C, 70.28; H, 12.35; P, 5.66.

Di-*n*-hexadecyl hydrogen phosphate (13a): mp 74–75 °C (lit.[30] mp 74–75 °C).

Sodium Salts of 1–13. General Procedure. A solution of sodium ethoxide (1.02 mmol) in anhydrous ethanol (10 mL) was added to a solution of the di-*n*-alkyl hydrogen phosphate (1.02 mmol) in ethanol (10 mL). Sometimes gentle heating was required to obtain a clear solution. If turbidity persisted (**2, 5, 11, 13**), the solution was filtered through a hard paper filter (MN673, Nagel and Com). Upon standing at −20 °C, the sodium salts crystallized. The material was removed by vacuum filtration and dried over P_2O_5. The sodium salt derived from **1** was crystallized from acetone–ether.

Vesicle Preparation. Vesicles formed from the sodium di-*n*-alkyl phosphates were prepared by the ethanol injection method.[31] Thus, the sodium di-*n*-alkylphosphate (10 mg) was dissolved in 100 μL of 96% ethanol. Using a preheated Hamilton microsyringe (60 °C), small aliquots of this solution (50 μL for fluorescence polarization measurements, 80 μL for electron microscopy or ^{31}P NMR spectroscopy) were injected into 5 or 2 mL, respectively, of a 5 mM Hepes/5 mM sodium acetate buffer (pH 7.4 with 0.1 N NaOH) which was kept at the required temperature ($>T_m$, see Table I). In all cases clear solutions were obtained.

Electron Microscopy. The samples were examined with a Philips EM 300 electron microscope operating at 80 kV. Carbon-coated Formvar grids, pretreated by glow discharge in air (sometimes 1-aminopentane) were used as matrices. Aliquots of vesicles solutions were stained with a 1% (w/v) solution of uranyl acetate. For cryo-electron microscopy, a film of the vesicle solution on carbon-coated grids was quenched frozen in liquid ethane using a guillotine-like apparatus. Liquid ethane prevented the formation of vapor films around the specimen during freezing. The grid was transferred to liquid nitrogen, mounted in a Philips PW6591 cooling holder, and rapidly introduced into a Philips EM400 electron microscope. The temperature was kept below −140 °C to avoid crystallization of the vitrified buffer. Thin sections[17] of the samples were fixed with 2.5% solutions of glutaraldehyde and post-fixed with 1% osmium tetroxide for 2 h. Fixed samples were dehydrated, embedded, sectioned, and then stained with uranyl acetate.

Fluorescence Polarization. These measurements were performed with a SLM Aminco SPF 500C spectrofluorometer equiped with a thermostated cell holder. DPH (5×10^{-8} M) was excited at 360 nm; the emission wavelength was 428 nm. The degree of fluorescence polarization (P) was calculated from

$$P = \frac{I_\parallel - I_\perp}{I_\parallel + I_\perp}$$

where $I_\parallel$ and $I_\perp$ are the fluorescence intensities determined with the polarizers oriented parallel and perpendicular, respectively, to the direction of polarization of the excitation radiation. The value of $I_\perp$ was corrected for the intrinsic polarization of the instrument. The amphiphile concentration was 5×10^{-5} M.

^{31}P NMR Spectroscopy. Linewidths were measured (at 121.4 MHz, Varian VXR 300 spectrometer) as a function of temperature using freshly prepared vesicle solutions containing 10% (w/w) D_2O as a lock solvent. Measurements were always initiated at the highest temperature. The time interval between two measurements was ca. 15 min.

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