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New Amphiphiles: 2',3'-Di-*O*-acyl-5'-nucleotides

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Synopsis

2',3'-Di-*O*-acyladenosine-5'-phosphate and 2',3'-di-*O*-acyl-uridine-5'-phosphate (acyl = palmitoyl and stearoyl) were synthesized from the corresponding nucleosides. These lipids were converted readily into well-defined liposomes (diameter, 200-1000Å) upon sonication in water. The liposomal membranes having the palmitoyl and stearoyl groups showed a main phase transition in a temperature range of 42-45 and 51-57 ℃, respectively.

KEYWORDS: Amphiphile, liposome, membrane, nucleotide, phospholipid

Introduction

Various amphiphiles have been known to produce bilayer assemblies such as the liposomes.[1] The vesicles accommodating various substances in the hydrophobic membrane and/or the aqueous interior have been studied in pharmaceutical, cosmetic and other fields.[2,3] Lipid components of those vesicles include: natural lipids, chemically modified natural lipids, and synthetic products.[4,5] We wish to describe here the synthesis of a new type of phospholipid (**1-4** of Scheme 1) starting from nucleosides.

Results and Discussion

Although various lipoid compounds bearing nucleic acid-related moieties have been reported, many of them were the 1,2-*O*-diacyl (or dialkyl)glycerols, which were attached to nucleic acid-bases, nucleosides and nucleotides driectly, or derivative(s) of the nonnatural double chain-lipophilic compounds connected with the nucleic acid-parts.[6-11] By contrast, **1-4** are the 5'-nucleotide derivatives having long chain-acyl groups at the 2'-*O* and 3'-*O* positions.

The nucleotide-lipids could be prepared easily by a series of well-known reactions as displayed in Scheme 1. The following procedure is typical. Palmitoyl chloride (3.6 g, 13 mmol) was added to an ice-cooled chloroform solution (30 ml) of 5'-*O*-trityluridine (3.0g, 6.2 mmol) and anhydrous pyridine (5 ml). After stirring magnetically overnight at room temperature, the reaction mixture was washed with water, dried over sodium sulfate and concentrated to give the residue, which was stirred subsequently with 5% acetic acid-HBr (25 ml) to precipitate trityl alcohol immediately. The reaction mixture was filtered and fractionated between chloroform and water. The organic layer was washed with water, dried and concentrated to afford 2',3'-di-*O*-palmitoyluridine, which was recrystallized from chloroform; 1.7 g (38%);m.p. 109-112 ℃; R_f 0.51 (chloroform-methanol, 15:1 v/v). Next, the di-*O*-acylurdine (0.20 g, 0,28

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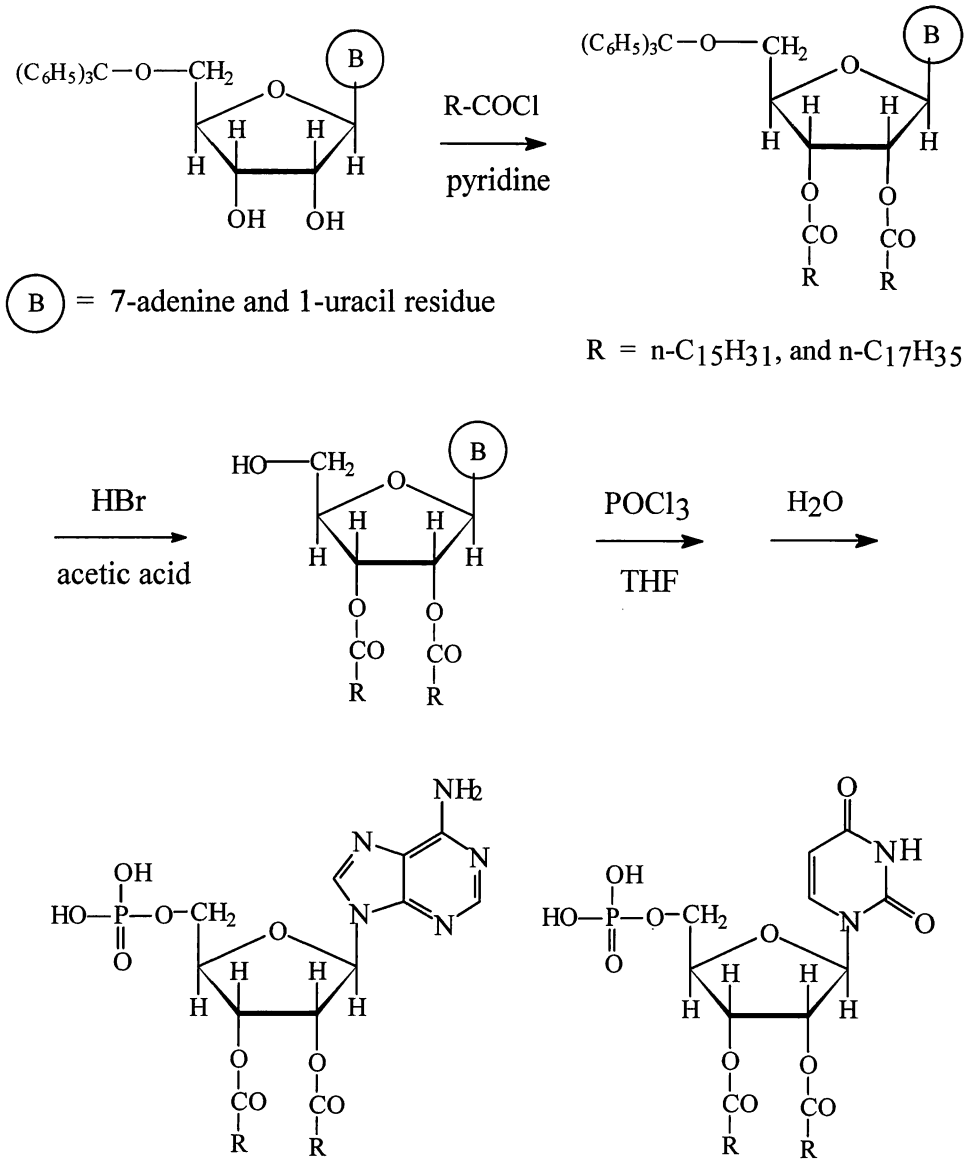
mmol) in THF was allowed to react with phosphoryl chloride (2 ml) at room temperature for a day, cooled and stirred with 0.1 M potassium chloride (10 ml) for a few hours. The resulting reaction mixture was fractionated between water (pH 2 by hydrochloric acid) and chloroform. The organic layer was concentrated to give the



Scheme 1.

residue which was applied to the gel column (Sephadex LH-20) equilibrated with a mixture of chloroform-methanol (2:1 v/v). Elution with the same solvent provided 2',3'-di-*O*-palmitoyluridine-5'-phosphate (**1**) as a colorless solid, 76 mg (34%). Table 1 displays the overall yields, physical constants, etc. of the nucleotide-lipids prepared.

The compounds (**1-4**) were found to give readily rise to organic assemblies upon sonication in water. Namely, a suspension of the lipids in pure water (1-2 mg/ml) was treated by means of a prove-type sonicator at 120 W per 1 cm^2 of a cross sectional area of a titan tip and at 55 ℃ (for the lipids having palmitoyl chains) or 65 ℃ (for the lipids having stearoyl chains) for 5 min x 6 times. The resulting transparent aqueous suspensions were centrifuged at 2000 g for 10 min, and the resulting supernatant were subjected to

Table 1. Yields [a, b] and physical constants [c, d] of various nucleotide-lipids (**1-4**), and Tm of the liposomal membranes.

1	yield, 18%; m.p. 157-160 ℃; R_f 0.15; λmax258 nm (ϵ12600 in THF); δ 0.87 (two t, 6H, $2CH_3$), 6.25 (d, 1H, J=6.5 Hz, 1'-H), 8.19 (s, 1H, 2-H) and 8.58 (s, 1H, 8-H); FABMS m/e (rel. intensity) 822 $(M-1)^-$ (80); T_m, 45 ℃, ΔH, 6.4 kcal/mole.
2	yield, 20%; m.p. 163-166 ℃; R_f 0.20; λ_{max} = 256 nm (ϵ11900 in a mixture of methanol and THF, 4:1 v/v); δ 0.87 (complex m, 6H, $2CH_3$), 6.20 (d, 1H, J=6.5 Hz, 1'-H), 8.08 (s, 1H, 2-H) and 8.54 (s, 1H, 8-H); FABMS m/e (rel. intensity) 880 $(M-1)^-$ (7); T_m, 57 ℃,ΔH, 8.1 kcal/mole.
3	yield, 21%; m.p. 57-62 ℃; R_f0.06; λ_{max} = 257 nm (ϵ8700 in a mixture of methanol and THF, 4:1 v/v); δ 0.85 (m, 6H, $2CH_3$), 5.49 (d, 1H, 1'-H, J=63 Hz), 6.00 (m, 1H, 5-H) and 7.78 (m, 1H, 6-H); FABMS m/e (rel. intensity) 799 $(M-1)^-$ (50); T_m, 42.6 ℃,ΔH, 6.3 kcal/mol.
4	yield, 24%; m.p. 93-97 ℃; R_f0.10 ; λ_{max} = 258 nm (ϵ7600 in methanol-THF = 4:1 v/v); δ 0.88 (m, 6H, $2CH_3$), 5.50 (d, 1H, 1'-H, J=33 Hz), 5.90 (m, 1H, 5-H) and 7.63 (m, 1H, 6-H); FABMS m/e (rel. intensity) 855 $(M-1)^-$ (45); T_m, 51.3 ℃, ΔH, 6.8 kcal/mol.

[a] All of the nucleotide-lipids in the table had the elemental analysis (C, H and N) agreed with the assigned structure within ±0.3 %. [b] The overall synthesis-yield on the basis of the used amounts of adenosine or uridine. [c] The solvent systems used in TLC was chloroform-methanol-concentrated ammonia = 65:30:3 v/v/v. [d] The 400 MHz ^{1}H-NMR spectra were measured by means of a Jeol GX-400 spectrometer using a dilute solution in $CDCl_3$ and CD_3OD (2:1 v/v) and tetramethylsilane as an internal standard. FABMS spectra were taken by a Jeol HX-100 spectrometer in a negative ion mode. Samples in glycerol as a matrix was subjected to a beam of xenon atom produced at 8 kV and 2 mA. The main transition temperature (Tm) was obtained from heating curves recorded with a Rigaku DSC-8230 differential scanning calorimeter (lipid concentration = 8-9 mg/ml) by the procedure similar to that mentioned previously.[13]

transmission electron microscopy to show small unilamellar or multilamellar vesicles. Typical micrographs are displayed in Figure 1. A light scattering study of the aqueous suspension suggested 200-1000 Å as a size distribution of the vesicles. Sonication of the lipid in an aqueous solution of 5(6)-carboxyfluoresceine followed by gel column chromatography (GPC) using Sephadex G-50 using water as a solvent [13] furnished an aqueous suspension of the liposomes containing the dye in the initial yellow band, which was well separated from the latter band of untrapped dye. The liposomes were formed quantitatively; viz., an GPC-eluent containing the liposomes was freeze dried to give 80-95% weight of the used amounts of the nucleotide-lipids. While the liposomal membranes of **1-4** were similar in a main phase transition temperature (T_m) to the membranes of the corresponding phosphatidylcholines, the formers were smaller in the ΔH than the latter (Table 1); c.f., the liposomal membranes of 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine and the distearoyl isomer had a main transition temperature of 41.4 and 54.9 ℃ and ΔH of 8.7 and 10.6 kcal/mole, respectively. The low ΔH values of the membranes of **1-4** has not been explained

well, but it might be caused by a restricted rotation between 2'-C and 3'C atoms to disrupt the regular packing of the neighboring chains. The liposome morphology of **1-4** was preserved at least for several hours at ambient temperature. A 1:1-5 molar mixture of **1-4** and an ordinary lipid such as egg lecithin afforded well defined and more stable liposomes. The use of the nucleotide-lipids has been under study.

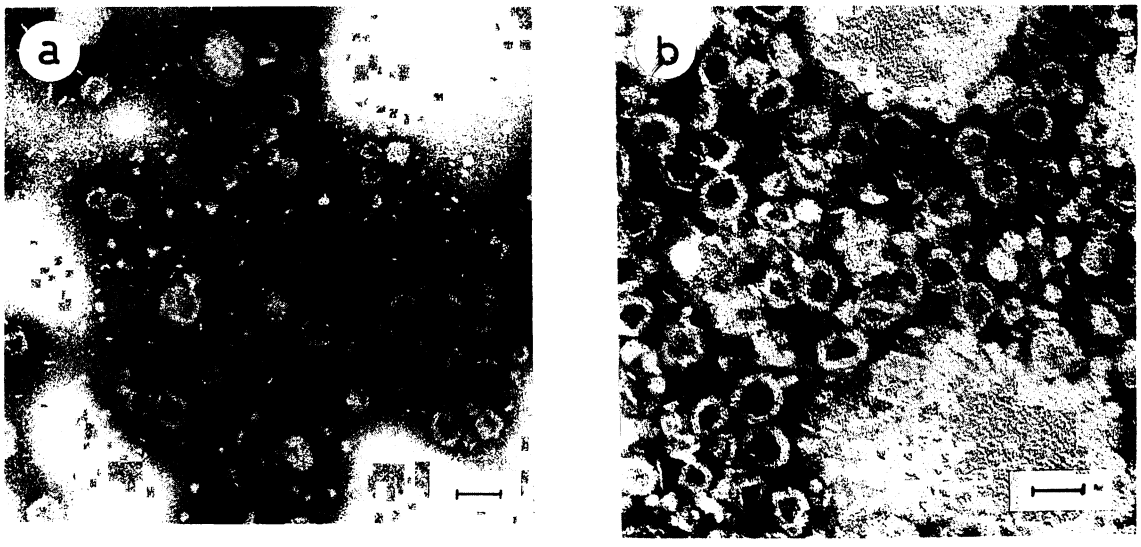


Figure 1. Transmission electron micrographs; negatively stained by phosphotungstic acid/NaOH (pH 7): **a** and **b** from **1** and **3**, respectively. A bar is 1000 Å.

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