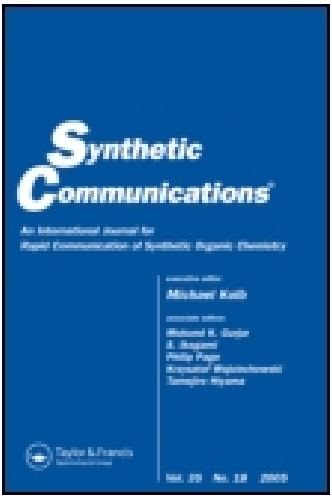

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Synthesis of Novel Nucleolipid Amphiphiles

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SYNTHESIS OF NOVEL NUCLEOLIPID AMPHIPHILES

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ABSTRACT: Three derivatives of uridine, thymidine and adenosine with one or two stearoyl chains, and three kinds of lipids with one or two nucleic acid bases were synthesized, which can form a stable monolayer at the air-water interface.

Molecular recognition by way of multiple hydrogen bonding is of great importance to the biochemical process because mutual recognition of complementary nucleic acid bases is a key factor for the reproduction and generation of cells [1]. Many studies have been performed to simulate the mutual recognition of nucleic acid bases in the replication process of DNA occurring in cells in a monolayer system formed by nucleic acid base-containing lipids [2-5]. The modeling studies of biological membranes should have important bearing on related processes occurring at surfaces of the biological molecular system. Furthermore, nucleic acid base derivatives with

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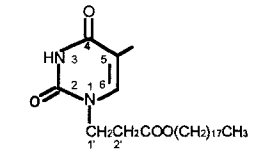
lipophilic groups have shown promising in anti-virus and anti-tumor activity[6,7]. So the synthesis and behavior of nucleoside surface-active derivatives in monolayers are significant for the preparation of new types of drugs and drug delivery systems [8]. Investigation indicated that all the nucleo-lipid amphiphiles synthesized in this paper can form stable monolayers on the air-water interface [5], and the details of recognition with complementary bases of nucleic acids dissolved in the subphase is in further studies.

The chemical structures of the new nucleolipid amphiphiles synthesized in this paper are shown in Scheme 1.

Scheme 1: Chemical structures of the nucleolipid amphiphiles



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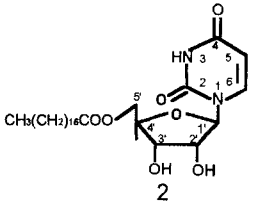

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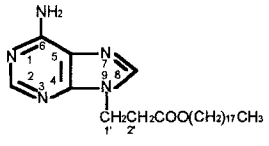

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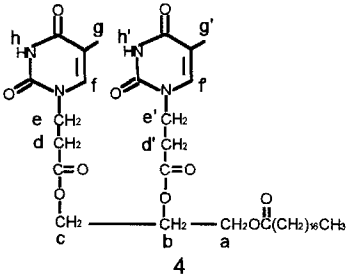

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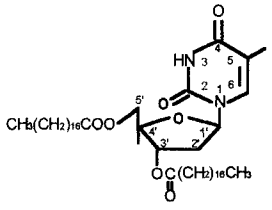

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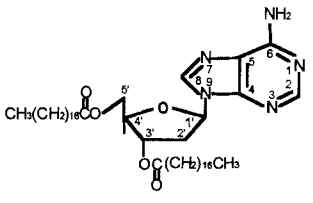


6

The crucial problem of their synthesis is the linkage of the hydrophilic (head) group to the hydrophobic (tail) chain through an ester bond. Two synthetic routes were adopted in this paper:

Route 1.

$$RCH_2CH_2COCl + R'OH \rightarrow RCOOR'$$

Product	R	R'	Yield (%)
1	Thy (C-1)	$CH_3(CH_2)_{16}CH_2$	56
2	$CH_3(CH_2)_{13}CH_2$	Urd (C-5')	60

Route 2.

$$RCH_2CH_2COOH + R'OH \xrightarrow{DCC,DMAP} RCH_2CH_2COOR'$$

Product	R	R'	Yield (%)
3	Ad (N-9)	$CH_3(CH_2)_{16}CH_2$	81
4	Thy (N-1)	$CH_3(CH_2)_{16}COOCH_2$	74
5	$CH_3(CH_2)_{15}CH_2$	T (C-3', C-5')	75
6	$CH_3(CH_2)_{15}CH_2$	Ade (C-3', C-5')	78

The former reaction is faster but accompanied by side-reactions. Synthetic route 2 requires milder conditions, and has been shown to be an efficacious method for amino-containing nucleic acid bases used as the head group of the amphiphiles. DCC (dicyclohexylcarbodiimide) is used as dehydrant and DMAP (4-dimethylaminopyridine) as catalyst in Route 2, which makes the reaction more selective and higher yielding.

EXPERIMENTAL

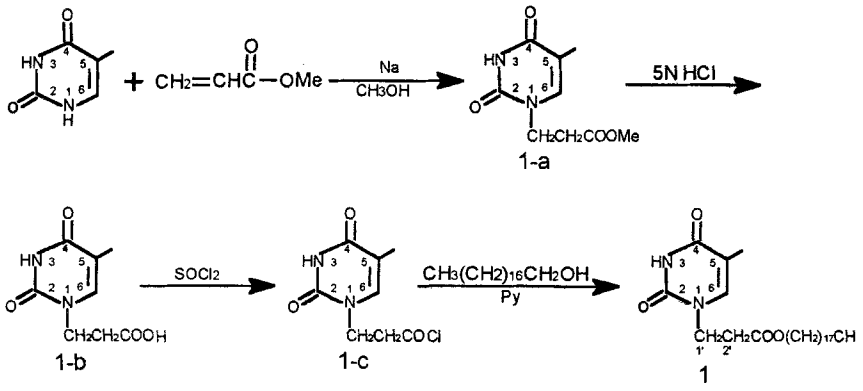
All the synthetic reactions are run at the same scale. The silica-gel (100-200 mesh) column chromatography used in purification are also the

same for all the reactions. Thin-layer chromatography (TLC) detection were taken on silica-gel (GF254, for TLC use) TLC-plate with Ultraviolet indicator. ^1H NMR spectra were recorded on a Bruker AM-500 spectrometer with deuterated chloroform as the solvent. UV spectra were taken on a Shimadzu UV-3100 spectrometer in chloroform. Elemental analyses were performed on a Perkin-Elmer 240C analyzer.

1-(2-Carboxyethyl) thymine **1** (typical procedure)

1-β-Carboxyethyl thymidine (**1-b**) was obtained via the method reported [9] . 1-β-Carboxyethyl thymidine (0.12g, 0.61 mmol) and thionyl chloride (0.2mL, 2.76 mmol) were heated together, under reflux (bath temp., 85°C) for 1 hour. Then the excessive thionyl chloride was removed by evaporation (35 mmHg. 30°C). 0.5 mL dry pyridine and octadecanol (0.14g,

Scheme 2: Synthesis of 1-(2-Carboxyethyl) thymine **1**



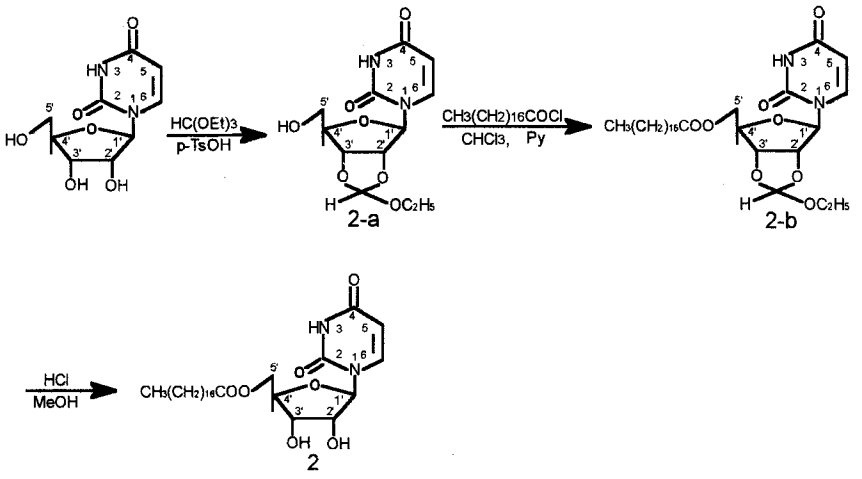
0.52mmol) were added to the reaction mixture, then stirred for 12 hours at room temperature. 3 mL distilled water and 20 mL chloroform were added to the reaction mixture. The organic layer was desiccated with anhydrous sodium sulfate, then after being concentrated and purified via silica-gel

column chromatography, eluted with 2% acetone-chloroform and then 5% acetone-chloroform, to give 0.131g 1-(2-carboxyethyl) thymine in 56% yield.

5'-Stearoyl-uridine **2** (typical procedure)

2',3'-O-ethoxymethylidneuridine (**2-a**) was prepared by the method reported [10,11]. 2',3'-O-ethoxymethylideneuridine (0.20 g, 0.66 mmol) and octadeconoyl chloride (0.20g, 0.66 mmol) are dissolved in dry chloroform (20 mL) and a small amount of dry pyridine (about 1.0 mL), and the reaction mixture stirred for 24 hours at room temperature. After evaporation of the

Scheme 3: Synthesis of 5'-Stearoyl-uridine **2**

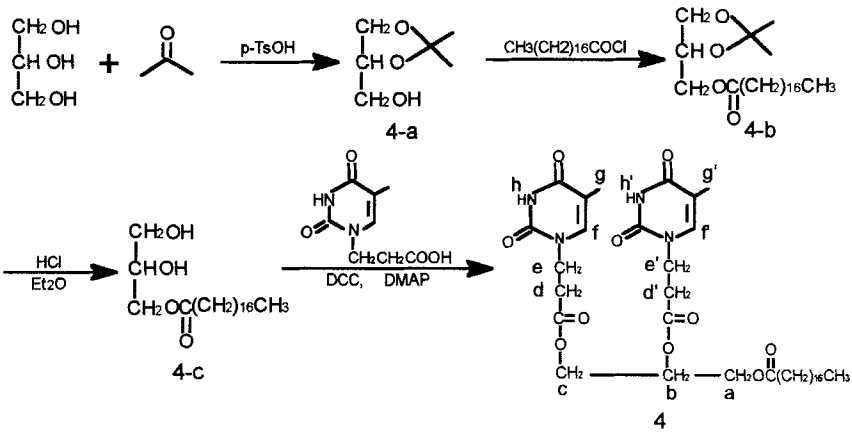


solvent, the residue was purified by silica-gel column chromatography using chloroform, then 3% methyl alcohol-chloroform as eluent. Pure (2',3'-O-ethoxymethylidene-5'-stearoyl)-uridine (**2-b**) from the chromatography was dissolved in methyl alcohol (10 mL) and hydrochloric acid (12 *M*, 1mL) and stirred for 1 hour at room temperature. Then the solution was carefully

neutralized with aqu. ammonia. The reaction mixture was concentrated, the residue washed with chloroform and dissolved in DMF; solids which did not dissolve were removed by filtration. The filtrate was concentrated and the residue washed with ion-free water, abs. ethyl alcohol and dry ethyl ether, and then desiccated in vacuum drying oven (20 mmHg, 60°C). Then 0.198g 5'-stearoyl-uridine was given in 60% yield.

Nucleolipid **4** (typical procedure)

Scheme 4: Synthesis of Nucleolipid **4**



Isopropylidene glycerol (**4-a**) and 1-β-carboxyethyl thymidine were obtained by means of methods reported elsewhere [9,12]. Isopropylidene glycerol (2.6 g, 19.8 mmol) was dissolved in dry pyridine, and octadeconoyl chloride (5.8 g, 19.2 mmol) was slowly added dropwise to the solution which was cooled to about 0°C. The reaction mixture was stirred for 24 hours in an ice-bath. The solid product **(4-b)** obtained after evaporating the reaction solution (10 mmHg, 50°C) was recrystallized twice from methanol.

Ester **4-b** was dissolved in hydrochloric acid (12 *M*, 10 mL) and ethyl ether (10 mL). The solution was cooled in an ice-bath, and stirred until the solution become transparent. Then the solvents were evaporated (10 mmHg, 50°C), and ester **4-c** (0.18 g, 0.5mmol), 1-β-carboxyethyl thymidine (0.30 g, 1.5 mmol), DCC (0.36 g, 1.7 mmol), and a catalytic amount of DMAP were dissolved in dry pyridine (4 mL). The mixture was stirred at room temperature for three days. About 0.5 mL distilled water was added to the reaction mixture to quench the reaction mixture. The precipitate which proved to be the hydrous product of DCC (dicyclohexylurea), was removed by filtration. Chloroform (20 mL) was added to the filtrate and then water. The organic layer was concentrated and purified via silica-gel column chromatography, eluted with 10% acetone-chloroform then 20% acetone-chloroform. Therefore 0.266g pure nucleolipid 4 was got in 74% yield.

Compound **1**:

mp: 100-101°C. ^{1}H NMR 500 MHz ($CDCl_3$): 8.86 (s, 1H, N-H); 7.19 (s, 1H, H-6); 4.08 (t, 2H, H-1'); 3.96 (t, 2H, CH_2 (1)); 2.76 (t, 2H, H-2'); 1.91 (s, 3H, T- CH_3); 1.67 (m, 2H, CH_2 (2)); 1.60 (m, 2H, CH_2 (3)); 1.29-1.25 (m, 28H, CH_2 (4-17)); 0.87 (t, 3H, CH_3 (18)). Anal: Calc. for $C_{26}H_{46}O_4N_2$: C, 69.28; H, 10.31; N, 6.22. Found C, 69.97; H, 10.78; N, 6.19. UV ($CHCl_3$): λ_{max}, 270 nm; λ_{min}, 235 nm. TLC (SiO_2 / methanol : chloroform = 1:9): R_f = 0.77.

Compound **2**:

mp: 172-173°C. ^{1}H NMR 500 MHz ($CDCl_3$): 11.38 (s, 1H, N-H); 7.62 (d, 1H, H-6); 5.75 (t, 1H, H-1'); 5.65 (d, 1H, H-5); 4.25-4.16 (m, 2H, H-5'); 4.06 (m, 1H, H-2'); 3.98 (m, 1H, H-3'); 3.93 (m, 1H, H-4'); 2.32 (t, 2H, CH_2 (1)); 1.51 (m, 2H, CH_2 (2)); 1.23 (m, 28H, CH_2 (4-17)); 0.85 (t, 3H,

CH_3 (18)). Anal: Calc. for $C_{26}H_{46}O_7N_2$: C, 62.61; H, 9.32; N, 5.62. Found C, 63.13; H, 9.74; N, 5.59. UV ($CHCl_3$): λ_{max}, 256 nm; λ_{min}, 228 nm. TLC (SiO_2 / methanol : chloroform = 1 : 9): $R_f = 0.58$.

Compound **3**:

mp: 105-107°C. ^{1}H NMR 500 MHz ($CDCl_3$): 8.34 (s, 1H, H-2); 7.99 (s, 1H, H-8); 6.39 (broad) NH_2; 4.52 (t, 2H, H-1'); 4.06 (t, 2H, CH_2 (1)); 2.93 (t, 2H, H-2'); 1.64-1.24 (m, 32H, CH_2 (2-17)); 0.87 (t, 3H, CH_3 (18)). Anal: Calc. for $C_{26}H_{45}O_2N_5$: C, 67.92; H, 9.89; N, 15.24. Found C, 68.30; H, 10.18; N, 15.16. UV ($CHCl_3$): λ_{max}, 258 nm; λ_{min}, 224 nm. TLC (SiO_2 / acetone : chloroform = 1 : 1): $R_f = 0.48$.

Compound **4**:

mp: 67-68°C. ^{1}H NMR 500 MHz ($CDCl_3$): 8.96 (s, 2H, H-h, h'); 7.17 (s, 2H, H-f, f'); 5.23 (m, 1H, H-b); 4.23 (m, 2H, H-c); 4.12 (m, 2H, H-a); 3.96 (m, 4H, H-e, e'); 2.79 (m, 4H, H-d, d'); 2.30 (t, 2H, CH_2 (2)); 1.91 (s, 6H, H-g, g'); 1.61 (m, 2H, CH_2 (3)); 1.59 (m, 2H, CH_2 (4)); 1.26 (m, 26H, CH_2 (5-17)); 0.88 (t, 3H, CH_3 (18)). Anal: Calc. for $C_{37}H_{58}O_{10}N_4$: C, 61.81; H, 8.15; N, 7.79. Found C, 62.36; H, 8.41; N, 7.76. UV ($CHCl_3$): λ_{max}, 266 nm; λ_{min}, 232 nm. TLC (SiO_2 / acetone : chloroform = 3 : 7): $R_f = 0.32$.

Compound **5**:

mp: 84-85°C. ^{1}H NMR 500 MHz ($CDCl_3$): 8.27 (s, 1H, N-H); 7.28 (s, 1H, H-6); 6.31 (m, 1H, H-4'); 5.21 (m, 1H, H-3'); 4.42-4.30 (m, 2H, H-5'); 4.23 (m, 1H, H-4'); 2.46 and 2.13 (m, 2H, H-2'); 2.34 (m, 4H, CH_2 (2)); 1.93 (s, 3H, T-CH_3); 1.64-1.25 (m, 60H, CH_2 (3-17)); 0.87 (t, 6H, CH_3 (18)). Anal: Calc. for $C_{46}H_{80}O_7N_2$: C, 71.44; H, 10.45; N, 3.62. Found C, 72.17; H,

10.84; N, 3.39. UV ($CHCl_3$): λ_{max}, 266 nm; λ_{min}, 238 nm. TLC (SiO_2 / acetone : chloroform = 1 : 9): $R_f = 0.75$.

Compound **6**:

mp: 76-77°C. ^1H NMR 500 MHz ($CDCl_3$): 8.36 (s, 1H, H-2); 8.00 (s, 1H, H-8); 6.43 (m, 1H, H-1'); 5.74 (broad) NH_2; 5.42 (m, 1H, H-3'); 4.42-4.34 (m, 3H, H-4', 5'); 2.91 and 2.62 (m, 2H, H-2'); 2.34 (m, 4H, CH_2 (2)); 1.68 (m, 4H, CH_2 (3)); 1.62 (m, 4H, CH_2 (4)); 1.25 (m, 52H, CH_2 (5-17)); 0.88 (t, 6H, CH_3 (18)). Anal: Calc. for $C_{46}H_{80}O_5N_5$: C, 70.44; H, 10.30; N, 8.93. Found C, 71.07; H, 10.72; N, 9.36. UV ($CHCl_3$): λ_{max}, 258 nm; λ_{min}, 239 nm. TLC (SiO_2/methanol : chloroform = 1 : 9): $R_f = 0.69$.

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