

# Surface Properties of Novel Surfactant, Dihexadecyl Gemini Phosphate, Monolayers on Water Surface by Dropping Method

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#### Abstract

We have synthesized first novel dihexadecyl phosphate (DHGP-n) gemini surfactants containing alkyl chain (( $C_nH_{2n}$ ); n = 3, 4, 5, and 6) spacers. Surfactants were used to form monolayers on water surface. Surface tension measurement (STm), Brewster angle microscopy (BAM), and density functional theory (DFT) calculation were used to investigate these monolayers. Surface pressure ( $\pi$ ) and molecular area (A);  $\pi$ -A isotherm curves of all DHGP-n gemini surfactants showed a gradual increase in surface pressure without any break points in the curves, confirmed the formation of liquid-expand (LE) type monolayers. BAM observations also supported the STm results. Limited molecular area  $(A_0)$  of these monolayers depended on the spacer of gemini surfactants without spacer n = 4. Longer alkyl chain spacer in the series had higher value of  $A_0$  in  $\pi$ -A isotherm plots. In the series,  $A_0$  of spacer n = 4 was smallest among  $A_0$  values of other spacers. Density functional theory (DFT) method calculation also confirmed  $\pi$ -A isotherm curves pattern of these surfactants. Calculation showed that both odd and even numbers and length of the alkyl chain spacer influenced the structure of DHGP-n monolayer formation on the water surface.

### Keywords

Dihexadecyl Gemini Phosphates, Monolayers, Surface Tension Measurement, Brewster Angle Microscopy, Density Functional Theory Calculation

#### **1. Introduction**

Amphiphilic compounds including phosphate group such as phospholipids are fundamental components of many creatures. It is well known that these phospholipids are closely related with biological phenomena such as substance transportation and molecular recognition in living bodies [1]. Monoalkyl phosphate (MAP, **Figure 1(a)**) is an important candidate for investigation and elucidation of the above phenomena similar to two alkyl chains phospholipid such as dipalmitoyl phosphatidyl choline (DPPC) commonly and commercially known as lecithin. Moreover, the MAP has a characteristic of a dibasic acid, thus it is considered in the perspective of an anionic surfactant [2] [3] [4] [5] [6]. High yield production of monoalkyl phosphate was done by Kurosaki [7] and Imokawa *et al.* [8] and they conducted safety evaluation to promote and utilize basic detergents for body washer, metal corrosion inhibitor, catalyst for chemical reactions, and antistatic agents in resin treatment.

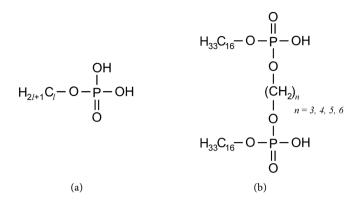
Among various surfactants, "gemini" surfactant essentially has a structure in which two general or common category monosurfactants having one hydrophobic and one hydrophilic moiety are connected by a spacer group such as an alkyl chain or a peptide group [9] [10] [11]. In aqueous solution of a gemini surfactant, Krafft point (Kp) and critical micelle concentration (CMC) are lower and the interfacial activity is higher compared to the corresponding monosurfactant. Therefore, gemini surfactants are more superior surfactants, thus several researchers [9] [10] [11] [12] have investigated them thoroughly. Moreover, gemini surfactants have two hydrophobic alkyl chains, thus they are better candidates to prepare and investigate model membranes for applications in drug delivery systems (DDS) [12] [13] [14].

In this research, to increase the scope and applications of gemini surfactants, we have synthesized a novel anionic gemini surfactant, dihexadecyl phosphates (DHGP-n (n = 3, 4, 5, and 6)) as shown in **Figure 1(b)**. DHGP-n has acted as biological substances for model biomembranes. We prepared DHGP-n monolayers on water surface and studied morphology of monolayer using surface tension measurement (STm) and Brewster angle microscopy (BAM). Monolayers were prepared by dropping method [15]. Structural optimization of DHGP-n molecules was performed by density functional theory (DFT) method and results were discussed at molecular level to justify the morphology of these monolayers.

#### 2. Materials and Methods

#### 2.1. Materials

Chemicals: 1-Hexadecanol ( $C_{16}H_{33}OH$ , 99%), phosphoryl chloride (POCl<sub>3</sub>, >99%), diethylether ( $C_2H_5$ -O- $C_2H_5$ , >99%), tetramethyl ammonium hydroxide-5H<sub>2</sub>O ((N(CH<sub>3</sub>)<sub>4</sub>OH-5H<sub>2</sub>O), >97%)), (methanol, >99%), (Chloroform, >99%) were obtained from Wako Pure Chemical Industries Ltd., Osaka,



**Figure 1.** Molecular structure of (a): monoalkyl phosphate (MAP) and (b) dihexadecyl Gemini phosphates (DHGP-n, n = 3, 4, 5, 6).

Japan. Purified water with conductance < 0.07  $\mu$ S/cm was used to prepare monolayer. Water was purified using super water purifying system (WL-21P; Yamato Scientific Corp. Ltd., Tokyo, Japan). Di-bromoalkane (Br-C<sub>n</sub>H<sub>2n</sub>-Br, n = 3, 4, 5, and 6, >97%) of mentioned spacer lengths were from Tokyo Chemical Industry Corp., Ltd., Tokyo, Japan.

#### 2.2. Synthesis of Compounds

### 2.2.1. Synthesis of Gemini Dihexadecyl Phosphates (DHGP-*n*,) with *n* = 3, 4, 5, and 6

Novel DHGP-*n* (n = 3, 4, 5, and 6) were synthesized by modifying synthesis methods of anionic phosphate surfactants [16] and dialkyl diphosphate [17].

#### 2.2.2. Synthesis of 1-Hexadecyl Phosphate ((1-HP, C<sub>16</sub>H<sub>33</sub>-O-PO(OH)<sub>2</sub>)

At first, starting material 1-hexadecyl phosphate (1-HP, **Figure 1(a)**) was synthesized. At room temperature, 1-hexadecanol ( $C_{16}H_{33}OH$ ) was reacted to phosphoryl chloride (POCl<sub>3</sub>) in solvent diethylether ( $C_2H_5$ -O- $C_2H_5$ ) thus product 1-hexadecyl dichloro phosphate ( $C_{16}H_{33}OPOCl_2$ ) was obtained. The product 1-hexadecyl dichloro phosphate then reacted with water in the same solvent to obtain 1-HP. The product was confirmed by nuclear magnetic resonance (NMR) and infrared spectroscopy (IR) recording.

#### 2.2.3. Synthesis of DHGP-n (n = 3, 4, 5, and 6)

Final products of dihexadecyl phosphates gemini surfactants series (DHGP-n,n = 3, 4, 5, and 6) (Figure 1(b)) were synthesized as following. N<sup>+</sup>(CH<sub>3</sub>)<sub>4</sub>)<sub>2</sub>(C<sub>16</sub>H<sub>33</sub>-O-P(O)(O<sup>-</sup>)<sub>2</sub> was obtained when 1-HP was reacted with tetramethyl ammonium hydroxide-5H<sub>2</sub>O in methanol at room temperature. Di-bromoalkanes

(Br-C<sub>n</sub>H<sub>2n</sub>-Br, n = 3, 4, 5, and 6) was reacted with N<sup>+</sup>(CH<sub>3</sub>)<sub>4</sub>)<sub>2</sub>(C<sub>16</sub>H<sub>33</sub>-O-P(O)(O<sup>-</sup>)<sub>2</sub> in the same solvent mixture and

 $N^+(CH_3)_4)_2(C_{16}H_{33}-O-P(O)(O^-)-O-C_nH_{2n}-O-P(O)(O^-)-O-C_{16}H_{33}$  was obtained. Finally, 36% hydrochloric acid was added to the solution to prepare

 $C_{16}H_{33}$ -O-P(O)(OH)-O- $C_nH_{2n}$ -O-P(O)(OH)-O- $C_{16}H_{33}$  (DHGP-*n*) series compounds. Products were again confirmed by NMR and IR spectroscopic methods.

#### 2.3. Apparatus Employed and Software Used

STm of monolayer was measured with Surface Tensiometer (CBVP-A3, Kyowa Interface Science Corp. Ltd., Tokyo). Brewster angle microscope used was of the model EMM633K; Filgen Inc., Nagoya, Japan. Image analysis of BAM data was carried out using USB-CAP type (SD-USB2CUP3; AREA Co. Ltd, Tokyo, Japan) imaging analysis software. A CCD camera, C5948-70; Hamamatsu Photonics, Hamamatsu, Japan, was used to detect reflected light from BAM microscope. Molecular area of surfactant (DHGP-*n*) molecules was calculated for experimental comparison by using a molecular model kit (MOL-TALOU Models, basic-A set, Koriyama, Japan).

#### 2.4. Monolayer Formation

Details of monolayer formation by dropping method have been reported previously [15]. In brief, 1 mM DHGP-*n* solution was prepared in chloroform. The solution was dropped and spread on the purified water surface using 100  $\mu$ l microsyringe (Ge-0583-04; Hamilton Co., Nevada, USA). The dropping was done at 1  $\mu$ l/min intervals. DHGP-*n*-chloroform droplet was dropped gently on water surface, next dropping was done only after the previous droplet was well expanded on the surface. The completion of monolayer formation was confirmed by the formation of lens of droplets observed on the water surface and the stable value of surface tension reached as described in the following section.

#### 2.5. Surface Tension Measurement (STm)

STm for each DHGP-*n* monolayer was measured with a Surface Tensiometer. The droplet of (DHGP-*n*)-chloroform solution was spread on the purified water surface. Wilhelmy plate dipped in the solution records the value of surface pressure in Surface Tensiometer when droplets were spread on the water surface. The surface pressure of each monolayer was recorded as a function of spreading volume at  $26.0^{\circ}C \pm 0.2^{\circ}C$ .

#### 2.6. Brewster Angle Microscopy (BAM)

The observation of BAM for visualizing the morphology of each DHGP-*n* monolayer was carried out using a Brewster angle microscope. A *p*-polarized light of 632.8 nm wavelength was irradiated at the Brewster angle of 53.1° on each monolayer surface and the reflected light was detected by a CCD camera. The lateral resolution of BAM was about 1  $\mu$ m. The formation process of each monolayer was directly observed *in situ* at arbitrary surface pressures: 2, 12, and 25 mN/m. The BAM measurement was conducted at 26°C ± 1°C and measurements were repeated three times for reproducibility.

#### 2.7. Density Functional Theory (DFT) Method Calculation with B3LYP/6-31G(d) Basis Set

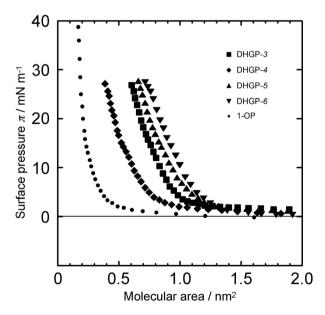
DFT calculations by B3LYP/6-31G (d) method have been carried out by using

the GAUSSIAN 03 program [18] for optimized molecular structure of DHGP-*n*. The initial structural parameters for geometry optimization were assumed to be  $60^{\circ}$  ( $-60^{\circ}$ ) (the *G*(*G*) conformation) torsional angles around two phosphate groups ( $C_{16}H_{33}$ -O-(P-O-)<sub>3</sub>,  $C_{16}H_{33}$ -O-(P-O-)<sub>3</sub> spacer), all-trans conformation for the other structures (alkyl chain and spacer), and the same direction of two alkyl chains.

#### 3. Results and Discussion

**Figure 2** shows surface pressure (SP,  $\pi$ ) versus molecular area (*A*) ( $\pi$ -*A*) isotherm curves of four DHGP-*n* (spacer length n = 3, 4, 5, 6) monolayers obtained at 26.0°C ± 0.2°C by using dropping method on the water surface. The horizontal axis represents *A*, calculated from molecular numbers in the dropping volume. Whereas vertical axis represents  $\pi$  plot when equilibrium reached after dropping DHGP-*n*-chloroform droplet and the SP value becomes constant. Isotherm curve of 1-octadecyl phosphate (1-OP, C<sub>18</sub>H<sub>35</sub>-O-PO(OH)<sub>2</sub>), synthesized by the scheme similar to 1-HP as in *Section* 2.2.2., has also been shown in **Figure** 2. Monolayer of 1-OP was obtained by using dropping method for comparison with DHGP-*n* surfactants. Isotherm of the monolayer formed on the water surface, can be explained in order as shown in the drawing (**Figure 2**) from the lower right to the upper left.

 $\pi$ -A isotherm curves formed on the monolayer of insoluble amphiphilic molecule at water surface is classified mainly into three categories [19] [20]. *First one* shows an extremely gradual increase of surface pressure and followed by sudden and steep increase in  $\pi$  with completion of monolayer formation. The shape of isotherm resembles with a typical titration curve. Such a monolayer is

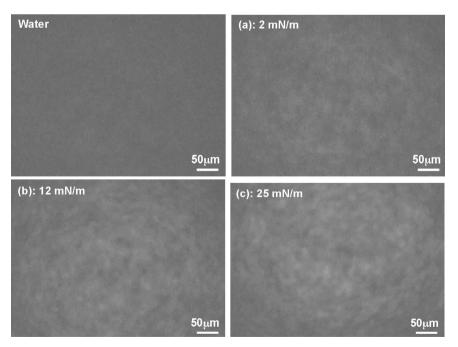


**Figure 2.** Surface pressure versus molecular area  $(\pi$ -A) isotherm curve of 1-OP and DHGP-n (n = 3, 4, 5, 16) at 26°C. •: DHGP-3, •: DHGP-4,  $\blacktriangle$ : DHGP-5,  $\blacktriangledown$ : DHGP-6, •: 1-OP.

an indication of existence of single liquid condensed (LC) state. Second one shows a gradual increase in  $\pi$  with monolayer formation. The shape of  $\pi$ -A isotherm is a monotonous curve without any break. Such a monolayer is an indication of existence of single liquid expanded (LE) state. *Third one* shows a mixed state of the above two changes in a  $\pi$ -A isotherm, at lower  $\pi$  values gradual increase in  $\pi$  is noticed, then there exists a LE-LC transition state with a plateau range in  $\pi$ , and followed by a steep increase in surface pressure, with monolayer formation. The shape of isotherm is a two steps curve.

Compared to the characteristics of the above general  $\pi$ -A isotherm curves, each DHGP-n compound studied in this research showed isotherm curve similar to a LE type monolayer.  $\pi$  increased gradually and monotonously up to 28 mN/m with completion of monolayer formation. The shapes of these isotherm curves were somewhat smooth but did not always resemble with a typical LE state such as dimyristoyl phosphatidyl choline (DMPC) monolayer [21] [22]. 1-OP had an intermediate isotherm curve between LE and LC states monolayers, a gradual increase in the beginning and subsequently a steep increase in  $\pi$  was noticed with monolayer formation at around 15 mN/m (Figure 2).

LE type of monolayer isotherms of DHGP-*n* molecules was also confirmed by the observation of BAM. Figure 3 shows BAM images of DHGP-6 monolayer at three  $\pi$ s (recorded in Figure 2): (a) 2 mN/m, (b) 12 mN/m, and (c) 25 mN/m. Figure 3 also shows the image of water surface as comparison. At 2 mN/m (Figure 3(a)), the image is in slight brighter contrast compared to that of water surface. Increasing surface pressure to 12 mN/m (Figure 3(b)), the image became brighter compared to Figure 3(a) and did not have a characteristic of lineand circle-type domain states as observed in the cholesterol and dipalmitoyl



**Figure 3.** BAM images of DHGP-6 monolayer on various surface pressure at 26°C. (a) 2 mN/m, (b) 12 mN/m, (c) 25 mN/m, including water surface.

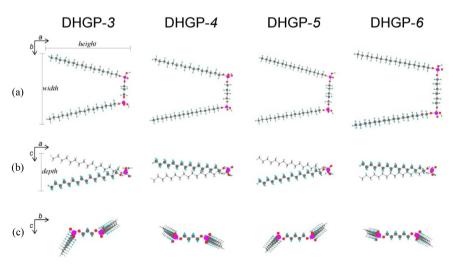
phosphatidyl choline (DPPC) monolayers [15]. The DHGP-6 monolayer was formed homogeneously. At 25 mN/m pressure (**Figure 3(c)**), the brightness of image increased further supporting homogeneous formation of DHGP-6 monolayer on water surface. Moreover, small circular-type 3D aggregates appeared in the image around high  $\pi$  (not shown). It was probably because at around the monolayer completion some splatter small lenses (chloroform solution) evaporated on the spot. Furthermore, almost same BAM images were obtained when other DHGP-*n* (*n* = 3, 4, 5) monolayers formation were investigated.

In LE state monolayer, it is generally known that the alkyl chain in the monolayer molecule contains either gauche structure or double bond, regardless of the number of alkyl chain in the molecule [20] [21] [23]. In case of gauche structure, especially the amphiphilic molecule with the carbon number of fourteen or less in alkyl chain forms LE state monolayer at room temperature. Actually, DMPC and 3,4-bis-tetradecylcarbonyl-hexanedioic acid (Gemsurf-14) both having carbon number fourteen formed LE state monolayers at 26°C, from our recent researches [22] [24]. Whereas the amphiphilic molecule with the carbon number sixteen or more in alkyl chain such as DPPC or Gemsurf-16 forms LC state monolayer around room temperature [20] [22] [24]. DHGP-n monolayer of the carbon number sixteen in alkyl chain was different from the above general tendency of molecules because if formed a LE type monolayer (Figure 2). It was also confirmed that two alkyl chains in DHGP-n molecules form almost all-trans structure at the completion of monolayer at  $\pi = 25$  mN/m as measured by infrared external reflection spectroscopy (IERS) [25], CH<sub>2</sub> anti-symmetric stretching vibration band  $(v_{as}(CH_2))$  for the methylene groups was observed around 2921 cm<sup>-1</sup>. Spatially free motion of two alkyl chains due to the existence of alkyl chain spacer contributes to the formation of LE type monolayer of DHGP-*n* molecules. For the same reason, LE state was observed in DPPC monolayer formation in the early stage of monolayer formation [15].

A remarkable point noticed in **Figure 2**, was the limiting molecular area  $(A_0)$  of DHGP-*n* monolayers. Under the present experimental conditions, the value of each  $A_0$  was 0.91 nm<sup>2</sup>, 0.71 nm<sup>2</sup>, 1.00 nm<sup>2</sup>, and 1.12 nm<sup>2</sup> for the compounds DHGP-3, DHGP-4, DHGP-5, and DHGP-6, respectively. Reproducibility of  $A_0$  was ±0.05 nm<sup>2</sup>, experiments were repeated twice for STm measurement. It is expected that the molecular area becomes large with increasing the length of alkyl chain spacer. Approximate calculation done by using molecular model kit described in *section* 2.3.also showed that the apparent molecular area of hydrophilic group (two phosphates and an alkyl chain spacer) of DHGP-*n* ( $A_{h-g}$ ) was estimated to be 0.40 nm<sup>2</sup>, 0.47 nm<sup>2</sup>, 0.54 nm<sup>2</sup>, and 0.61 nm<sup>2</sup> for DHGP-3, DHGP-4, DHGP-5, and DHGP-6, respectively. Whereas  $A_0$  of DHGP-*n* was becoming large by the order of 4, 3, 5, and 6.  $A_0$  of DHGP-4 was extremely small among all DHGP-*n*.  $A_0$  of 1-OP was 0.23 nm<sup>2</sup> and close to the value of LC monolayer of mono-alkyl amphiphilic molecule such as stearic acid [20].  $A_{h-g}$  of 1-OP was also 0.25 nm<sup>2</sup> and close to  $A_0$ .  $A_0$  of all DHGP-*n* molecules was more

than three times larger than that of 1-OP.

The specificity of  $A_0$  in each DHGP-*n* monolayer may have close relationship with the conformation of two hydrophilic phosphate groups and hydrophobic interaction between two alkyl chains (-C<sub>16</sub>H<sub>33</sub>-, -C<sub>16</sub>H<sub>33</sub>-) in DHGP-n. DFT calculations by B3LYP/6-31G(d) method have been carried out for the structural optimization of DHGP-n. These calculations have been performed with consideration that two alkyl chains in DHGP-n molecules orientate toward same direction similar to the molecular structure of monolayer on the water surface [18] [26]. Figure 4 shows the conformation of each DHGP-*n* molecule optimized by DFT calculations. The upper picture set (A) is the front of DHGP-n molecule, the middle (B) is the side of molecule, and the lower most (C) is the phosphate groups' side appearance. In Figure 4, three axes: a, b, and c defined are of DHGP-n molecules. From the initial condition that two alkyl chains orientate toward same direction allowing the interaction between two alkyl chains, the calculation results showed the possibilities: 1) two alkyl chains open outside on the a - b plane and these chain axes are not parallel (Figure 4(a)); 2) the alkyl chains are twisted and open opposite direction reciprocally from a-b plane (Figure 4(b)) and the degree of opening in DHGP-3 and -5 (odd spacers) is larger than DHGP-4 and -6 (even spacers) (Figure 4(b) and Figure 4(c)); 3) a zigzag plane of alkyl chain spacer is perpendicular to the *a-b* plane and parallel to the *b*-*c* plane (Figure 4(a) and Figure 4(c)). Moreover, torsional angles around two phosphate groups were GG (one side) and GG (another side) for DHGP-3 and -5 (odd spacers), and GG (one side) and GG (another side) for DHGP-4 and -6 (even spacers), respectively. Table 1 shows each value (height, width, and depth) of DHGP-n as inserted in DHGP-3 (Figure 4(a) and Figure **4(b)**, assuming that the structure of DHGP-*n* forms a rectangular parallelepiped. Calculated molecular area ( $A_{c-0}$ ) was 1.88, 1.28, 1.91, 1.75 nm<sup>2</sup> for DHGP-3, DHGP-4, DHGP-5, and DHGP-6, respectively. The order of  $A_{c.0}$  and size of



**Figure 4.** Conformation of DHGP-n (n = 3, 4, 5, 6) molecule optimized by DFT calculations. (a): front of DHGP-n molecule, (b): side of molecule, (c): phosphate groups side.

DHGP-n	DHGP-3	DHGP-4	DHGP-5	DHGP-6
Conformation	GGG'G'	GGGG	GGG'G'	GGGG
Height (nm)	2.00	2.00	2.00	2.00
Width (nm)	1.77	1.92	2.01	2.07
Depth (nm)	1.06	0.67	0.95	0.84
<i>Ac-o</i> (nm <sup>2</sup> )	1.88	1.28	1.91	1.75

**Table 1.** Each value (*height, width*, and *depth*) of DHGP-*n* as inserted in DHGP-3 of **Figure 3**. These values were measured by assuming that the structure of DHGP-*n* forms a rectangular parallelepiped.

molecular area did not necessarily agree with the experimental results because the calculation was performed on the condition of one molecule and DHGP-*n* molecule was in an ideal vacuum. In addition, initial calculation condition was also somewhat specific at high twist state near the hydrophilic group. Nevertheless, DFT calculation showed that only  $A_{c.0}$  of DHGP-4 was small compared to those of other DHGP-*n*.

In the case of carbon number of alkyl chain spacer is odd, each oxygen atom in two phosphates connecting to spacer both ends arranges the same side, thereby the degree of twist in two alkyl chains becomes larger for structural stabilization. Even number of spacer, on the other hand, makes the degree of twist smaller. The length of spacer also has an influence on the interaction between two alkyl chains. The longer is the length of spacer, the weaker is the interaction. These multiple effects would show the unique carbon number dependence of alkyl chain spacer on  $A_{c-0}$ . Therefore spacers' carbon number dependence on  $A_{c-0}$ . is the sum of these effects. The length of alkyl chain spacers in DHGP-n molecules forming monolayer on water surface is responsible for molecule's structure between all-trans and gauche forms [26] [27]. When the length of spacer becomes longer, the degree of twist of the spacer would become smaller for forming a monolayer. Due to restricted twist of spacer, the spacer would shrink and thus it strengthens the interaction between two alkyl chains leading to stable monolayer formation. Actually, shrinking of spacer was supported by recorded  $\pi$ -A isotherm, as experimental  $A_0$  of each DHGP-*n* was 30% to 50% smaller than those of calculated  $A_{c-0}$ . This anomaly, which  $A_0$  of DHGP-4 surfactant is smallest in the series though DHGP-3 spacer is shortest, has been interpreted considering the balance of the following factors: 1) conformation of each hydrophilic phosphate group restricted between alkyl chain and alkyl chain spacer; 2) length and structure of spacer and its even-odd effect; and 3) interaction between two alkyl chains.

Recent research by the electric conductivity measurement has shown that sodium-DHGP-4 salt  $(C_{16}H_{33}$ -O-P(O)(ONa)-O-C<sub>4</sub>H<sub>8</sub>-O-P(O)(ONa)-O-C<sub>16</sub>H<sub>33</sub>) synthesized from DHGP-4 had critical micellar concentration (CMC) of 0.071 mmol/kg in its water solution at 40°C. The CMC value was the same order concentration as other Gemini-type surfactant [9] [10] [11] [12] and also extremely small compared to general single chain anionic surfactants [27]. With the above result, we would like to expect that this novel DHGP-n would be not only utilized as new detergents and industrial products but also applied as model biomembrane substance and the functional substance such as solubilization surfactant for proteins.

#### 4. Conclusion

We have investigated the surface properties of the novel DHGP-n (n = 3, 4, 5, 6) compound's monolayer formed on the water surface at 26°C, using STm and BAM. It was found that all DHGP-n forms LE type monolayers. The limiting molecular area ( $A_0$ ) was increased in the order n = 4, 3, 5, and 6. Molecular area of compound n = 4 (four methyl spacer) was smallest in the series. DFT calculation also supported the smallest  $A_0$  of DHGP-4 among the series. DHGP-n surfactant compounds showed surface properties similar to other commercial and biosurfactants. These first synthesized novel gemini surfactants can be used as commercial detergents and model biomembrane forming substances.

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