Novel Globular Aggregates Composed of Synthetic Peptide Lipids

Yukito Murakami,* Jun-ichi Kikuchi, Toshihiko Takaki, and Katsuya Uchimura

Department of Organic Synthesis, Faculty of Engineering, Kyushu University, Fukuoka 812, Japan

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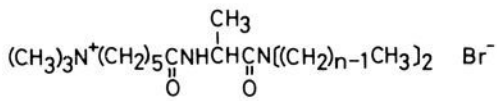
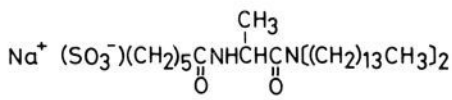
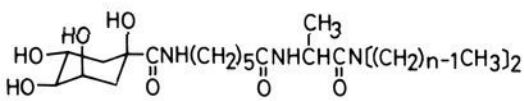
The aggregate morphology of biological lipids is primarily governed by their molecular structures and compositions of component lipids, and the bilayer aggregate is well-known as the most fundamental type of morphology. Meanwhile, the *lipidic intramembranous particles* have been recently found in a variety of lipid mixtures and in total lipid extracts under physiological conditions.[1] Although much attention has been focused on the nature of such lipidic particles in connection with the dynamic behavior of membranes such as fusion and fission, a lack of useful model membranes seems to have retarded physicochemical understandings of such aggregation behavior. In this paper, we report the formation of stable globular aggregates which appear in an aqueous dispersion of two kinds of peptide lipids having opposite charges, $N^+C_5Ala2C_{14}$[2] and $(SO_3^-)C_5Ala2C_{14}$.[3] Each lipid was



$N^+C_5Ala2C_n$ (n = 14,16)



$(SO_3^-)C_5Ala2C_{14}$



QC_5Ala2C_n (n = 14,16)

so designed as to attain an effective intramembrane interaction due to formation of the so-called hydrogen-belt domain[4] interposed between the hydrophobic region formed with aliphatic double chains and the hydrophilic zone with ionic head groups. We have shown previously that both of the present cationic and anionic lipids individually form bilayer membranes (vesicle and lamella) in the dispersion state and show the phase transition between gel and liquid crystalline states in the same temperature range (2.0 and 2.1 °C for $N^+C_5Ala2C_{14}$ and $(SO_3^-)C_5Ala2C_{14}$, respectively).[2,3]

Chloroform solutions of the cationic and anionic lipids were mixed at the equimolar ratio and evaporated to dryness. The residue was dispersed in deionized and distilled water (2.5 mM each of both lipids) by vortex mixing with glass beads at room temperature (ca. 20 °C), higher than the phase transition temperature of the lipid mixture (T_m, 4.9 °C). The ordered packing

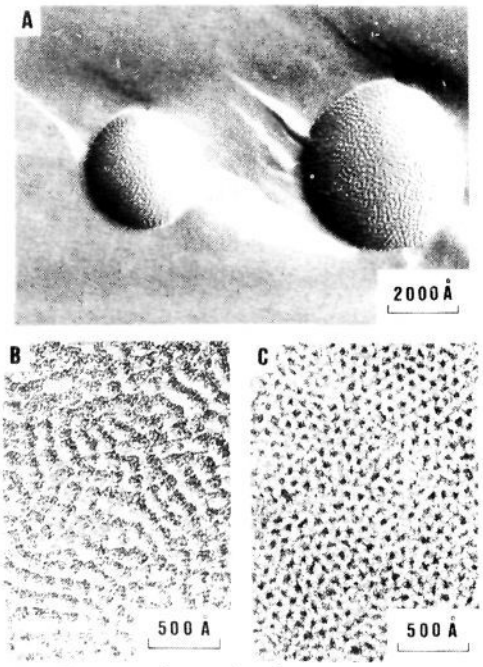


Figure 1. Electron micrographs for an equimolar mixture of $N^+C_5Ala2C_{14}$ and $(SO_3^-)C_5Ala2C_{14}$ (2.5 mM each) in the aqueous dispersion state, freeze fractured (A, B) and negatively stained with uranyl acetate (C), taken on a JEOL JEM-200B electron microscope installed at the Research Laboratory for High Voltage Electron Microscopy of Kyushu University.

assembly of lipidic particles was observed in electron micrographs of both freeze-fractured and negatively stained samples (Figure 1).[5] A repeating distance (95 ± 5 Å) for the network structure shown in Figure 1C corresponds to the magnitude of the thickness of two lipid monolayers (ca. 40 Å) plus the diameter of an inner aqueous compartment (ca. 55 Å) and is in good agreement with that evaluated from Figure 1B (110 ± 5 Å). The size of the inner aqueous compartment is considerably small as compared with those of the single-walled bilayer vesicles of individual lipids obtained by sonication of the aqueous dispersions (100–450 Å). This suggests that the intramembrane interaction among the ionic head groups is much stronger in the lipidic particles than those in the single-walled vesicles. The present aggregates provide largely developed domains (2000–20000 Å; see Figure 1A) as reflected on turbidity which are appreciably larger than those given by the individual bilayer membranes in the dispersion state. Nevertheless, the aggregate structure is so stable that any appreciable turbidity change was not detected for more than several weeks. The globular aggregate structure shown in Figure 1 is extremely sensitive to the lipid composition and subjected to spontaneous transformation into the normal bilayer aggregates when the composition loses fractional balance of the equimolar ratio to any slight extent as confirmed by electron microscopy with the negative staining technique and turbidity measurements. In addition, highly homogeneous mixing of the cationic and anionic lipids was attained at the equimolar ratio due to the electrostatic interaction among surface charges; the differential scanning calorimetry for an equimolar mixture of $N^+C_5Ala2C_{16}$ (T_m, 25.5 °C)[2] and $(SO_3^-)C_5Ala2C_{14}$ in the dispersion state showing a single and sharp phase transition peak at 15.5 °C.

The closely packed assembly of globular aggregates, schematically shown in Figure 2, definitely appears under the conditions that the electrostatic repulsion among polar head groups of lipid molecules is completely quenched. Moreover, we have previously found that nonionic peptide lipids bearing a quinoyl moiety as a head group, QC_5Ala2C_n (n = 14, 16),[3,6] form similar

(1) Verkleij, A. J. *Biochim. Biophys. Acta* **1984**, *779*, 43–63 and references cited therein.

(2) Murakami, Y.; Nakano, A.; Yoshimatsu, A.; Uchitomi, K.; Matsuda, Y. *J. Am. Chem. Soc.* **1984**, *106*, 3613–3623.

(3) Murakami, Y.; Kikuchi, J.; Takaki, T.; Uchimura, K.; Nakano, A. *J. Am. Chem. Soc.*, **1985**, *107*, 2161–2167.

(4) Brockerhoff, H. In "Bioorganic Chemistry"; van Tamelen, E. E., Ed.; Academic Press: New York, 1977; Vol. 3, Chapter 1.

(5) The freeze-fracture replicas were prepared on a Model FD-2A apparatus of Eiko Engineering Co. by courtesy of Professor T. Kunitake of our Department.

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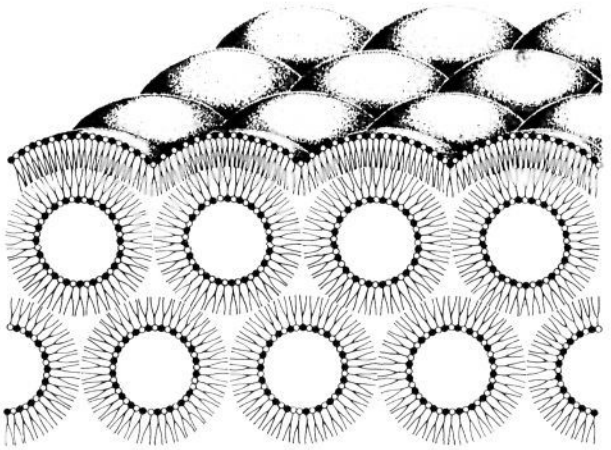

Figure 2. Schematic representation of the lipid aggregates formed with $N^+C_5Ala2C_{14}$ (O–⊂) and $(SO_3^-)C_5Ala2C_{14}$ (●–⊂) mixed at the equimolar ratio in water.

aggregates, in which an effective hydrogen-bonding interaction among head groups is presumably the predominant factor controlling the stabilization of such aggregates.

In conclusion, an attractive interaction among polar head groups of lipid molecules in aggregates is the primarily important factor for the formation of globular aggregates, and the subsequent hydrophobic interaction among these aggregates results in the closely packed arrangement. In addition, the hydrogen-belt domain in such aggregates may also be responsible for the morphological stabilization.

Registry No. $N^+C_5Ala2C_{14}$, 83825-02-9; $(SO_3^-)C_5Ala2C_{14}$, 95362-72-4.

(6) Murakami, Y.; Nakano, A.; Kikuchi, J.; Takaki, T.; Uchimura, K. *Chem. Lett.* **1983**, 1891–1894.

Regioselective Nucleophilic C-Alkylation of the Pyrrole Ring in an (η-Pyrrolyl)iodohydridorhenium Complex

Hugh Felkin* and Janusz Zakrzewski

Institut de Chimie des Substances Naturelles C.N.R.S., 91190 Gif-sur-Yvette, France

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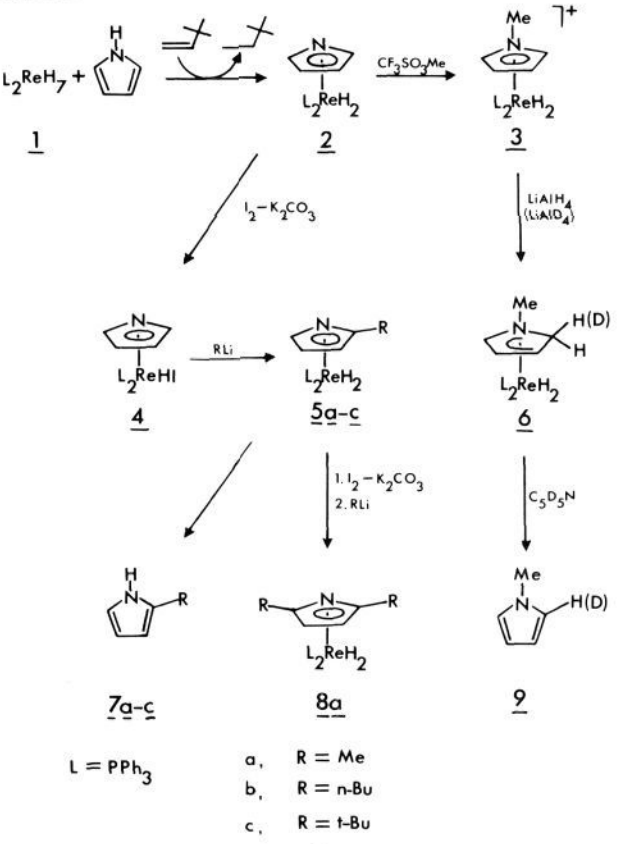
The electrophilic C-alkylation of pyrrole (as its magnesium salt) shows only modest selectivity for 2-substitution,[1,2] and C-alkylated pyrroles are generally prepared by less direct routes, including cyclization of acyclic precursors.[1] Here we report the preparation of an η-pyrrolyl iodo hydrido complex of rhenium **4** which undergoes *nucleophilic* C-alkylation in high yield (>90%) at the C-2 position of the pyrrolyl ligand. Whereas the activation toward nucleophiles of weakly nucleophilic organic molecules such as arenes by coordination to transition-metal centers offers one of the most useful synthetic strategies involving organotransition-metal complexes,[3] this kind of "Umpolung" does not seem to have been achieved previously in the case of the more nucleophilic aromatic nitrogen heterocycles.[4]

Scheme I



Our results are summarized in Scheme I (L = PPh_3). The η-pyrrolyl dihydrido complex **2**[5] was obtained in 61% yield by refluxing bis(triphenylphosphine)rhenium heptahydride (**1**)[6] with pyrrole (10 equiv) and 3,3-dimethylbutene (10 equiv) in THF (5 min).[7] Like pyrrole itself, this neutral complex reacts with

(1) Sundberg, R. J. In "Comprehensive Heterocyclic Chemistry"; Katritzky, A. R., Rees, C. W., Eds.; Pergamon Press: Oxford, 1984; Vol. 4, Chapter 3.06.

(2) (a) Skell, P. S.; Bean, G. P. *J. Am. Chem. Soc.* **1962**, *84*, 4655–4660. (b) Wang, N. C.; Teo, K. E.; Anderson, H. J. *Can. J. Chem.* **1977**, *55*, 4112–4116.

(3) Davies, S. G. "Organotransition Metal Chemistry: Applications to Organic Synthesis"; Pergamon Press: Oxford, 1982; Chapter 4. Collman, J. P.; Hegedus, L. S. "Principles and Applications of Organotransition Metal Chemistry"; University Science Books: Mill Valley, CA, 1980; Chapter 14. Semmelhack, M. F.; Garcia, J. L.; Cortes, D.; Farina, D.; Hong, R; Carpenter, B. K. *Organometallics* **1983**, *2*, 467–469 and references therein.

(4) Pannell, K. H.; Kalsotra, B. L.; Párkányi, C. *J. Heterocycl. Chem.* **1978**, *15*, 1057–1081. Bogdanov, G. M.; Bundel, Yu. G. *Khim. Geterosikl. Soedin.* **1983**, *8*, 1155–1172. Nucleophilic addition to (η-indole)$Cr(CO)_3$ has been investigated; in this case, however, the $Cr(CO)_3$ unit is coordinated to the carbocyclic ring and addition takes place on this ring: Semmelhack, M. F.; Wulff, W.; Garcia, J. L. *J. Organomet. Chem.* **1982**, *240*, C5–C10. Very recently, nucleophilic addition to a thiophene manganese cation has been reported: Lesch, D. A.; Richardson, J. W., Jr.; Jacobson, R. A.; Angelici, R. J. *J. Am. Chem. Soc.* **1984**, *106*, 2901–2906.

(5) All the organometallic complexes described are yellow (or orange, **4**) air-stable crystalline solids; they gave satisfactory elemental analyses (C, H, N, P) (except for the deuterio analogues, which were not analyzed). Selected NMR data: **2** ^1H (80 MHz, CD_2Cl_2) δ 5.43 (s, H2, H5), 4.53 (s, H3, H4), −10.07 (t, J = 41 Hz, ReH_2); $^{31}P\{Ar\ H\}$ (162 MHz, CD_2Cl_2) δ 33.61 (downfield from external H_3PO_4) (t, J = 41 Hz). **3** ^1H (80 MHz, acetone-d_6) δ 6.51 (s, H2, H5), 5.45 (s, H3, H4), 2.71 (s, Me), −8.00 (t, J = 41 Hz, ReH_2). **4** ^1H (80 MHz, CD_2Cl_2) δ 5.64 (s, H2, H5), 4.49 (s, H3, H4), −10.23 (t, J = 48 Hz, ReH). **5a** ^1H (200 MHz, C_6D_6) δ 5.47 (s, H5), 4.49 (d, J = 2 Hz) and 4.21 (d, J = 2 Hz) (H3, H4), 2.23 (s, Me), −9.52 (t, J = 40 Hz, ReH_2). **5b** ^1H (200 MHz, C_6D_6) δ 5.51 (s, H5), 4.58 (d, J = 2 Hz) and 4.22 (d, J = 2 Hz) (H3, H4), 2.52, 1.51, 1.23 (m, $(CH_2)_3$), 0.79 (t, J = 8 Hz, CH_3), −9.49 (t, J = 40 Hz, ReH_2). **5c** ^1H (80 MHz, C_6D_6) δ 5.38 (s, H5) 4.67 (d, J = 2 Hz) and 3.80 (d, J = 2 Hz) (H3, H4), 1.13 (s, t-Bu), −9.28 (t, J = 40 Hz, ReH_2). **6**-H ^1H (200 MHz, acetone-d_6) δ 5.22 (dt, J = 3.6, 5.4 Hz, H5-exo), 5.04 (s, H2), 3.66 (d, J = 3.6 Hz, H5-endo), 3.30 (d, J = 3.6 Hz) and 1.50 (d, J = 3.6 Hz) (H3, H4), 1.33 (s, Me), −2.0 (br) and −10.8 (br) (ReH_2). **8a** ^1H (200 MHz, C_6D_6) δ 4.28 (s, H3, H4), 2.23 (s, Me), −9.43 (t, J = 40 Hz, ReH_2).

(6) Chatt, J.; Coffey, R. S. *J. Chem. Soc. A* **1969**, 1963–1972. Baudry, D.; Ephritikhine, M.; Felkin, H. *J. Organomet. Chem.* **1982**, *224*, 363–376.

(7) For other reactions involving this L_2ReH_7 + olefin system, see: Baudry, D.; Daran, J. C.; Dromzee, Y.; Ephritikhine, M.; Felkin, H.; Jeannin, Y.; Zakrzewski, J. *J. Chem. Soc., Chem. Commun.* **1983**, 813–814 (furan). Baudry, D.; Ephritikhine, M.; Felkin, H.; Jeannin, Y.; Robert, F., *J. Organomet. Chem.* **1981**, *220*, C7–C10 (benzene). Baudry, D.; Ephritikhine, M.; Felkin, H.; Zakrzewski, J. *Tetrahedron Lett.* **1984**, *25*, 1283–1286 (*n*-alkanes). Baudry, D.; Ephritikhine, M.; Felkin, H.; Holmes-Smith, R. *J. Chem. Soc., Chem. Commun.* **1983**, 788–789 (cycloalkanes).

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