ACS ORGANIC & INORGANIC Au
AN OPEN ACCESS JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

pubs.acs.org/orginorgau

Article

Turn-On Fluorescent Probe Based on a Dansyl Triarginine Peptide for Ganglioside Imaging

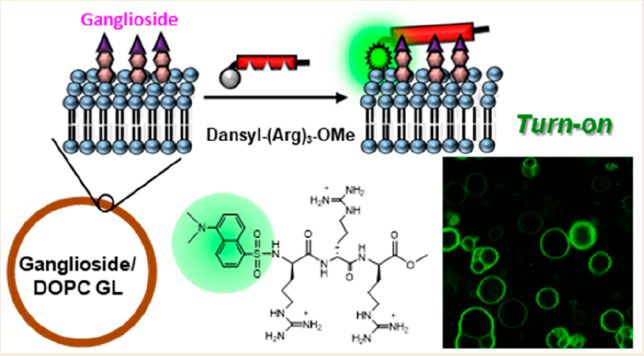
Kazunori Matsuura,* Koichi Hisamoto, Tomoya Tanaka, Ryota Sakamoto, Mizuki Okazaki, and Hiroshi Inaba

Cite This: *ACS Org. Inorg. Au* 2021, 1, 60–67

Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information

ABSTRACT: Gangliosides play pivotal biological roles in the animal cell membranes, and it is vital to develop fluorescent probes for imaging them. To date, various artificial receptors for ganglioside imaging have been developed; however, turn-on fluorescence imaging for gangliosides with high contrast has not been achieved. We developed a simple fluorescent probe on the basis of a dansyl triarginine peptide for turn-on ganglioside imaging on the liposome membrane. The probe bound to monosialyl gangliosides and other anionic lipids with association constants was 10^5 M^{-1}, which enhanced from 6-fold to 7-fold the fluorescence intensity. Upon binding to monosialyl ganglioside-containing giant liposomes, the turn-on probe selectively enhanced the fluorescence intensity compared with the other anionic lipids. This simple peptide probe for turn-on fluorescence imaging of gangliosides would provide a novel molecular tool for chemical biology.



KEYWORDS: *Gangliosides, Turn-on fluorescence probe, Dansyl triarginine, Fluorescence imaging, Giant liposome*

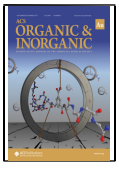
INTRODUCTION

Glycosphingolipids containing sialic acid residues, named "gangliosides", are essential components of all animal cell membranes and are particularly abundant on the plasma membranes of neurones.[1] Gangliosides on the cell membrane function as factors that control the immune system, nervous system, metabolic system, and cancer progression.[2,3] Changes in the amount and structure of gangliosides play essential roles in cancer infiltration, metastasis, and maintenance of cancer hepatocytes.[3] Additionally, gangliosides are involved in the differentiation of nerve cells and protein folding of neurodegenerative diseases, such as Alzheimer's.[4] Some viruses and pathogens exploit gangliosides as their receptors for infection to the host cell.[5]

Imaging gangliosides on cell membranes is a critical technique to understand and profile the function of gangliosides at the molecular level.[6] Fluorescence-labeled antiganglioside antibodies, lectins, and toxins were often used as probes for ganglioside distributions on cell membranes.[7−10] Since these fluorescent probes have a larger molecular size than gangliosides, there is a concern that accurate ganglioside distribution cannot be detected. As another approach, fluorescence-labeled synthetic gangliosides enabled single-molecular imaging with high spatiotemporal resolution.[11,12] However, this imaging is not for endogenous gangliosides, and it is necessary to add a fluorescence-labeled ganglioside from the outside.

Over the past two decades, various synthetic receptors with small molecular sizes to bind sugars in aqueous media have been developed.[13−21] Davis developed "synthetic lectins" on the basis of a water-soluble macrotricycle architecture, bound to specific sugars via CH−π and hydrophobic interactions with association constants on the order of 10^2 M^{-1} in aqueous solution.[13] Various molecular sensors have also been developed by molecular design on the basis of the interaction between phenylboronic acid and the *cis*-diol of sugars in aqueous media.[16,17] Mazik and co-workers developed acyclic artificial receptors binding *N*-acetylneuraminic acid (a kind of sialic acid) with association constants on the order of 10^3−10^5 M^{-1} in water−dimethyl sulfoxide solution.[18,19] Davis and co-workers developed a cationic receptor possessing 24 guanidinium units binding *N*-acetylneuraminic acids in an aqueous solution.[20] The fluorescence intensity of the cationic receptor was decreased as binding *N*-acetylneuraminic acid, and the analysis gave stepwise association constants K_1, K_2 = 1300, 790 M^{-1} in water. Matsubara and co-workers have also developed artificial peptide receptors that recognize ganglio-

Received: June 26, 2021
Published: August 10, 2021



https://doi.org/10.1021/acsorginorgau.1c00013
ACS Org. Inorg. Au 2021, 1, 60–67

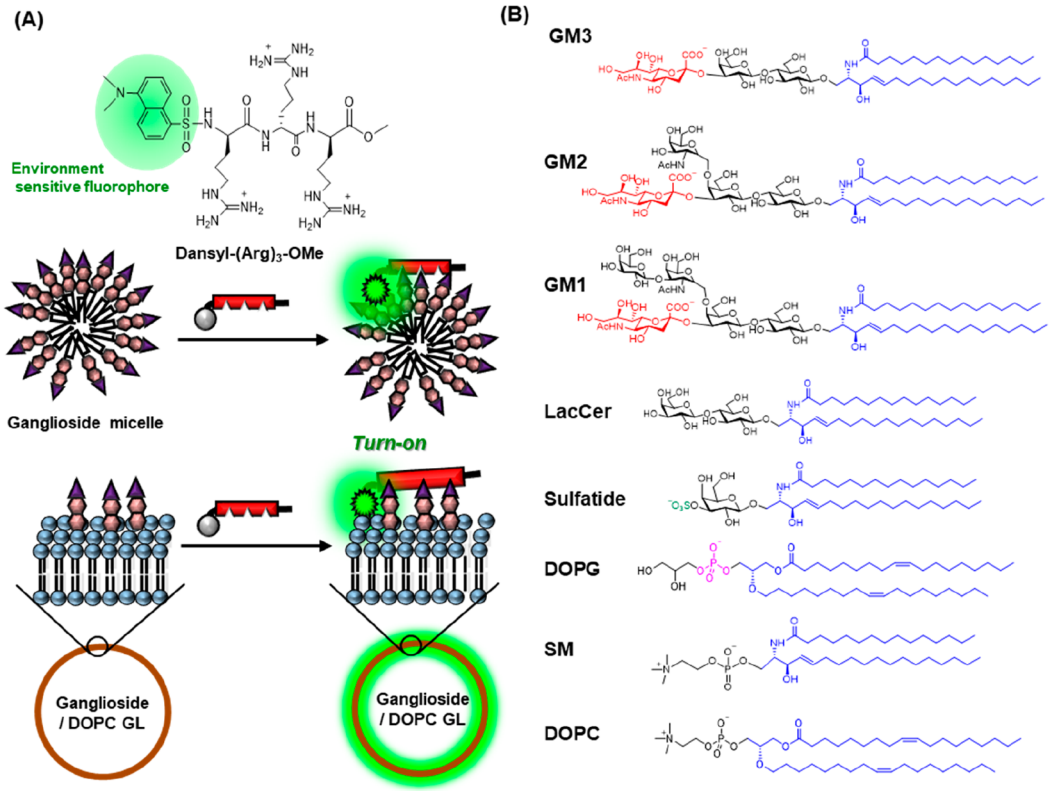
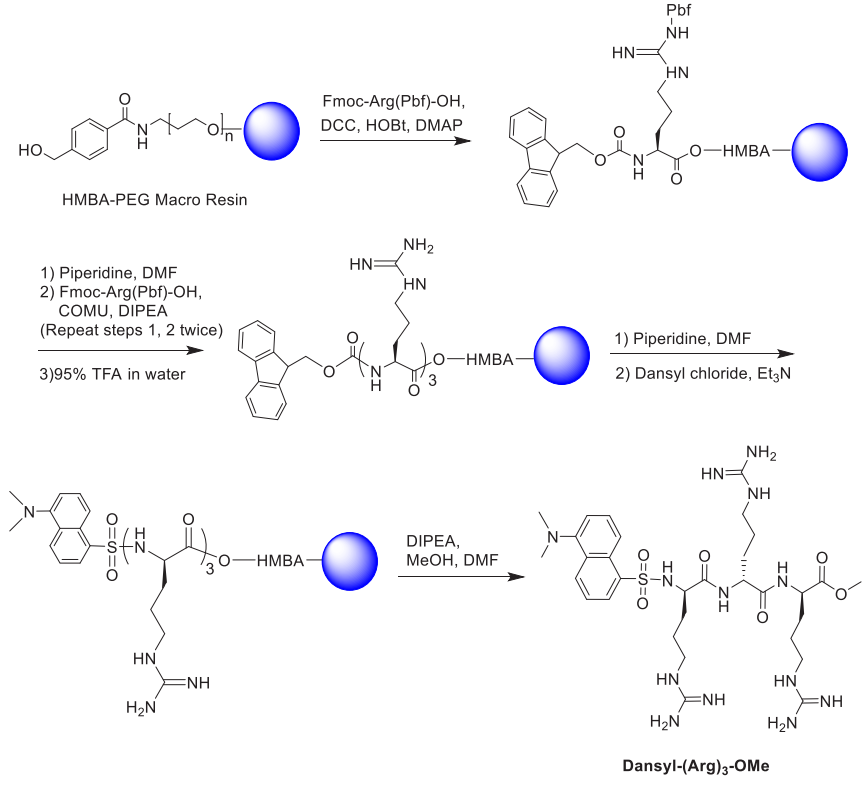


Figure 1. (A) Illustration of the fluorescence turn-on of dansyl-$(Arg)_3$-OMe on monosialyl ganglioside micelles and GL of DOPC containing monosialyl gangliosides. (B) Structure of lipids used in the study.

Scheme 1. Synthesis of Dansyl-$(Arg)_3$-OMe



sides.[22−27] For example, a peptide receptor binding to ganglioside monosialate 3 (GM3) with association constants on the order of 10^5 M^{-1} was selected by the phage display technique.[25,26] They recently demonstrated that a GM1-binding pentadecapeptide identified from a phage-displayed library is available as a molecular probe for specific ganglioside

https://doi.org/10.1021/acsorginorgau.1c00013
ACS Org. Inorg. Au 2021, 1, 60−67

nanoclustering sites in caveolae/membrane rafts on the cell surface.[27] Recently, fluorescence,[28−33] magnetic resonance,[34] and Raman imaging[35] of gangliosides on cell surfaces have been achieved by artificial receptors based on phenylboronic acid. Sellergren and co-workers demonstrated that sialic acid-imprinted fluorescent core−shell particles bound to cell surfaces possessing sialic acids with association constants on the order of 10^6 M^{-1}.[36]

This study developed a novel, simple turn-on fluorescent probe, dansyl-$(Arg)_3$-OMe peptide, to bind monosialyl gangliosides on giant liposomes (GLs) with relatively high affinity (Figure 1). Generally, environment-responding fluorescent chromophores, such as the dansyl group, enable high-contrast turn-on fluorescent imaging for target proteins.[37−39] Since the vicinity of the lipid bilayer membrane is a relatively low-polarity environment, it is expected that the fluorescence will turn on when a probe with a dansyl group binds to a ganglioside-containing membrane. As a simple binding motif to gangliosides on membranes, we employed triarginine peptide bearing guanidinium side chains. To date, it has been demonstrated that various artificial receptors possessing guanidinium cations recognize anionic guest molecules via electrostatic interactions and hydrogen bonds.[40−42] Although a 1:1 interaction between the guanidinium cation and carboxylate is weak (e.g., the lactate−guanidinium ion pair has a stability of only $K_a \leq 6$ M^{-1} in water), the multivalent arrangement of guanidinium can enhance electrostatic interactions and hydrogen bonds to anion guests.[42] Additionally, it has been demonstrated that hydrogen bonds and electrostatic interactions are significantly enhanced on the lipid bilayer rather than in bulk water.[43] Therefore, we expected that the dansyl-$(Arg)_3$-OMe peptide can bind to monosialyl gangliosides on a lipid bilayer with a sufficient affinity constant to turn on the fluorescence.

RESULTS AND DISCUSSION

Dansyl-$(Arg)_3$-OMe peptide was synthesized on 4-hydroxymethylbenzoic acid (HMBA)-modified resin by standard solid-phase Fmoc chemistry (Scheme 1). Fmoc-Arg(Pbf)-OH (Pbf = 2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulphonyl) was esterified on HMBA-modified resin with condensation reagents *N*,*N*′-dicyclohexylcarbodiimide (DCC), hydroxybenzotriazole (HOBt), and 4-*N*,*N*-dimethylaminopyridine (DMAP). After deprotection of the Fmoc group with piperidine, condensation of Fmoc-Arg(Pbf)-OH on the resin with (1-cyano-2-ethoxy-2-oxoethylidenaminooxy)-dimethylamino-morpholino-carbenium hexafluorophosphate (COMU) and *N*,*N*-diisopropylethylamine (DIPEA) was repeated for two cycles. After the deprotection of Fmoc and Pbf groups, dansyl chloride was reacted to the N-terminal amino group of peptidyl resin. The peptide was cleaved from the resin by methanolysis to afford the peptide methyl ester (isolated yield: 4.9%). The low yield is due to hydrolysis as the side reaction and insufficient deprotection of the Pbf group. The peptide was purified by reverse-phase HPLC (Figure S1) and confirmed by MALDI-TOF-MS, ESI-MS, and ^{1}H NMR (Figures S10−S12). Dansyl-Arg-OMe (isolated yield: 33%) and dansyl-$(Arg)_2$-OMe (isolated yield: 6.6%) peptides were also synthesized by the same protocol.

We conducted a fluorescence titration experiment to evaluate whether dansyl-$(Arg)_3$-OMe works as a turn-on fluorescent probe for monosialyl gangliosides. Figure 2A shows that adding an increasing amount of ganglioside monosialate 1 (GM1) to 1 μM dansyl-$(Arg)_3$-OMe in 10 mM HEPES buffer (pH 7.0) significantly increased the fluorescence intensity at 520 nm. Similarly, the increasing addition of ganglioside monosialate 2 (GM2) and GM3 also showed a significant increase in the fluorescence intensity of dansyl-$(Arg)_3$-OMe (Figure S2A,B). These monosialyl gangliosides formed micelles up to 100 μM, as shown by dynamic light scattering (Figure S4). Thus, it appears that dansyl-$(Arg)_3$-OMe binds to monosialyl ganglioside micelles to enhance the fluorescence intensity because of the lower polar environment. The normalized fluorescence intensity was plotted against the concentration of lipids (Figure 2B), and the curve was treated with the Langmuir equation to determine the association constant K_a and the maximum increment of normalized fluorescence intensity $\Delta I_{max}/I_0$ as summarized in Table 1 (see also Figure S3). Dansyl-$(Arg)_3$-OMe showed a 6-fold to 7-fold enhancement of the fluorescence intensity upon binding to all monosialyl gangliosides (GM3, GM2, and GM1). Interestingly, the association constants to monosialyl gangliosides were on the order of 10^5, which are comparable with that of sialylgalactose-binding peptides selected from a phage library (K_d ~10^{-6} order).[26] These surprisingly large association constants appear to be the cause of the enhanced electrostatic interactions and hydrogen bonds at the lipid assemblies.[43] Other anionic lipids, sulfatide, and 1,2-dioleoyl-*sn*-glycero-3-phospho-*rac*-(1-glycerol) (DOPG) were also bound to dansyl-$(Arg)_3$-OMe with a K_a of 10^5 order (Figure 2B, Figure S2C,D, and Table 1). The increment of fluorescence intensity by adding sulfatide was smaller than that of monosialyl gangliosides and DOPG. By contrast, adding

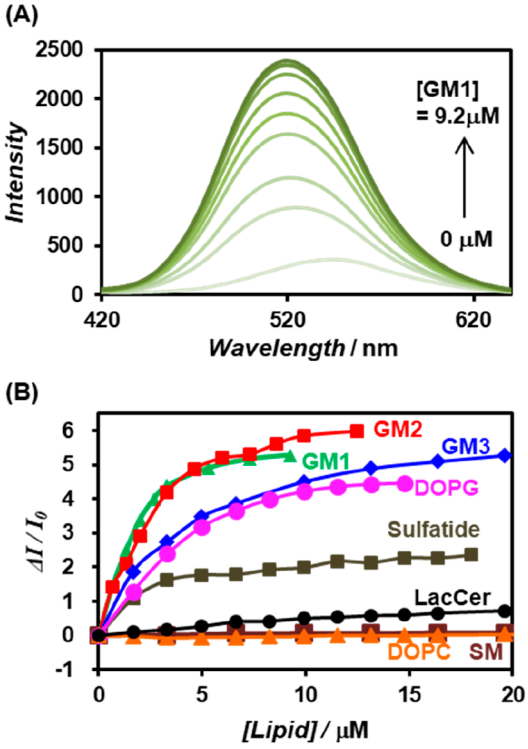


Figure 2. (A) Changes in fluorescence spectra (Ex = 330 nm) of 1 μM Dansyl-$(Arg)_3$-OMe with an increasing addition of 0−9.2 μM GM1 in 10 mM HEPES buffer (pH 7.0) at 25 °C. (B) Dependence of the concentration of lipids on the normalized fluorescence intensity of dansyl-$(Arg)_3$-OMe at 520 nm in 10 mM HEPES buffer (pH 7.0) at 25 °C.

https://doi.org/10.1021/acsorginorgau.1c00013
ACS Org. Inorg. Au 2021, 1, 60−67

Table 1. Binding Parameters for the Interactions of Dansyl-$(Arg)_n$-OMe (n = 1−3) with Lipids in 10 mM HEPES Buffer (pH 7.0) at 25°C[a]

	dansyl-$(Arg)_3$-OMe		dansyl-$(Arg)_2$-OMe		dansyl-Arg-OMe	
lipids	K_a/M^{-1}	$\Delta I_{max}/I_0$	K_a/M^{-1}	$\Delta I_{max}/I_0$	K_a/M^{-1}	$\Delta I_{max}/I_0$
GM3	$(2.3 \pm 0.1) \times 10^5$	6.4 ± 0.1	$(8.9 \pm 0.6) \times 10^3$ ($n = 3.7 \pm 0.3$)	4.2	$(1.3 \pm 0.2) \times 10^4$ ($n = 3.6 \pm 0.3$)	5.1
GM2	$(3.4 \pm 0.3) \times 10^5$	7.6 ± 0.2	$(2.0 \pm 0.3) \times 10^4$	9.5 ± 0.5	$(1.6 \pm 0.3) \times 10^4$ ($n = 3.7 \pm 0.8$)	4.8
GM1	$(4.5 \pm 0.6) \times 10^5$	6.8 ± 0.3	$(4.1 \pm 0.6) \times 10^4$	4.7 ± 0.3	$(1.3 \pm 0.2) \times 10^4$ ($n = 2.8 \pm 0.5$)	6.1
sulfatide	$(4.4 \pm 0.5) \times 10^5$	2.6 ± 0.7				
DOPG	$(1.9 \pm 0.2) \times 10^5$	6.3 ± 0.3				

[a]The Hill coefficient is indicated by n.

a zwitterionic lipid, sphingomyelin (SM) or DOPC, did not affect the fluorescence intensity of dansyl-$(Arg)_3$-OMe (Figure 2B and Figure S2E,G). The addition of lactosylceramide (LacCer), which is a sialic acid-deficient structure from GM3, minimally affected the fluorescence intensity (Figure 2B and Figure S2F). These results suggest that dansyl-$(Arg)_3$-OMe was selectively bound to anionic lipids via electrostatic interaction.

We next questioned whether this fluorescence enhancement of dansyl-$(Arg)_3$-OMe was selective for anionic lipids but not anionic polysaccharides. Figure 3 and Figure S5 show that

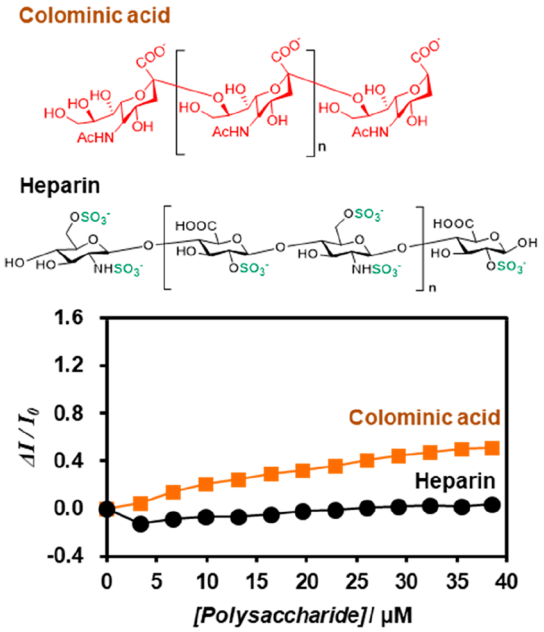


Figure 3. Dependence of the concentration of colominic acid and heparin on the normalized fluorescence intensity of dansyl-$(Arg)_3$-OMe in 10 mM HEPES buffer (pH 7.0) at 25 °C.

adding colominic acid (polysialic acid, M_w: 30 kDa) gently increased the fluorescence intensity of dansyl-$(Arg)_3$-OMe, whereas heparin (M_w: 15 kDa) possessing sulfonate anions minimally affected the fluorescence intensity. The K_a of binding of colominic acid to dansyl-$(Arg)_3$-OMe was calculated to be $(2.2 \pm 0.3) \times 10^2$ M^{-1} using the molarity of the sialic acid unit, and the $\Delta I_{max}/I_0$ was 1.2 ± 0.1 (Figure S5C). These control experiments support that dansyl-$(Arg)_3$-OMe was selectively bound to anionic lipids to enhance fluorescence intensity. Both the anionic function and hydrophobic moiety are probably important for the strong binding and enhancement of the fluorescence intensity of dansyl-$(Arg)_3$-OMe.

To confirm the effect of the number of arginine residues on the turn-on sensing, we examined the changes in fluorescence spectra of dansyl-$(Arg)_2$-OMe and dansyl-Arg-OMe with the increasing addition of monosialyl gangliosides (Figure 4 and

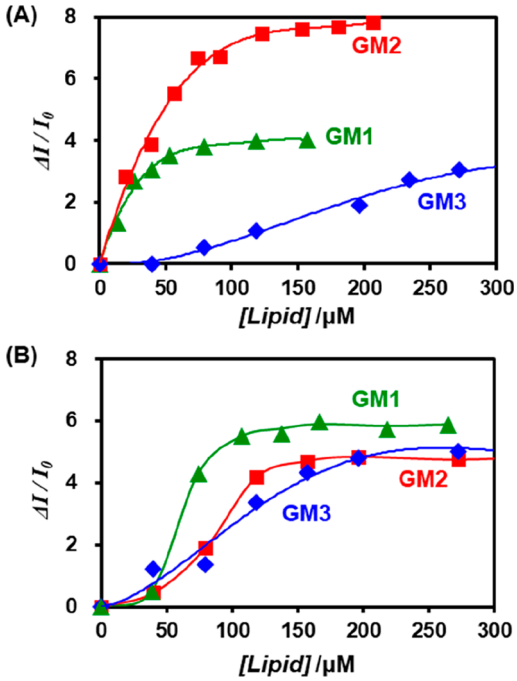


Figure 4. Effect of number of Arg. Dependence of the concentration of monosialyl gangliosides on the normalized fluorescence intensity of dansyl-$(Arg)_2$-OMe (A) and dansyl-Arg-OMe (B) in 10 mM HEPES buffer (pH 7.0) at 25 °C.

Figures S6 and S7). These dansyl-peptides required a higher concentration (>50 μM) of monosialyl gangliosides than dansyl-$(Arg)_3$-OMe to increase the fluorescence intensity. As some binding curves showed sigmoidal concentration dependence, we treated the sigmoidal binding curves with the Hill equation to determine the K_a and Hill coefficient n (Figure S8, Table 1). These behaviors indicate that some kind of cooperative effect was worked upon the binding to monosialyl gangliosides, although it is difficult to explain clearly the cause. The K_a of monosialyl gangliosides binding to these dansyl-peptides was calculated to be approximately 10^3−10^4 M^{-1}, which was significantly smaller than that of dansyl-$(Arg)_3$-OMe (Table 1). These results indicate that dansyl-$(Arg)_3$-OMe enhanced the affinity to assemblies of anionic lipids by multivalent binding between Arg and anionic groups. The effect of the concentration of dansyl-$(Arg)_3$-OMe on the fluorescence intensity in the presence of 10 μM GM1 micelles

https://doi.org/10.1021/acsorginorgau.1c00013
ACS Org. Inorg. Au 2021, 1, 60−67

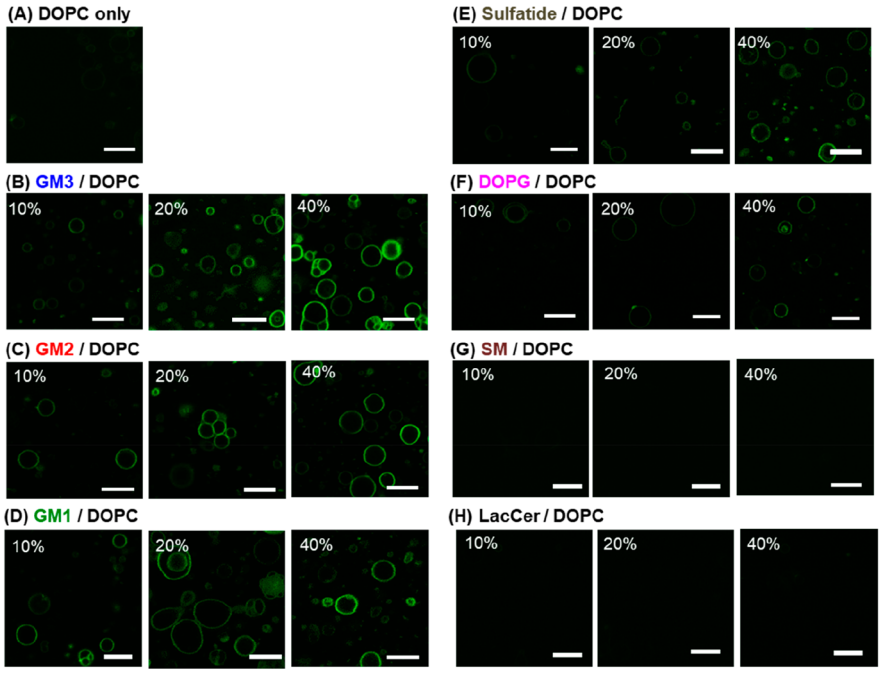


Figure 5. CLSM images (scale bar: 10 μm) of GLs of DOPC (A) containing 10−40% GM3 (B), GM2 (C), GM1 (D), sulfatide (E), DOPG (F), SM (G), and LacCer (H), stained with 10 μM dansyl-(Arg)3-OMe in 10 mM HEPES buffer (pH 7.0) at 25 °C.

showed that the intensity was saturated at the concentration above 5 μM (Figure S9). Thus, we employed 10 μM dansyl-$(Arg)_3$-OMe for a turn-on fluorescent probe for monosialyl ganglioside imaging on GLs.

The monosialyl ganglioside-containing GL of 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC) was prepared via a natural swelling method using D-glucose.[44] An aqueous solution of dansyl-$(Arg)_3$-OMe in 10 mM HEPES buffer (pH 7.0) was added for a final concentration of 10 μM to an aqueous dispersion of monosialyl ganglioside-containing GLs with a diameter of 3−10 μm. Then, the GLs were imaged by confocal laser scanning fluorescence microscopy (CLSM). Figure 5 shows CLSM images of GLs of DOPC containing 0−40% monosialyl gangliosides or other lipids, stained with 10 μM dansyl-$(Arg)_3$-OMe. The relative fluorescence intensity of dansyl-$(Arg)_3$-OMe on each GL was quantitatively estimated from the CLSM images by subtracting the background intensity and was plotted against the lipid contents in GL (Figure 6A). When 10 μM dansyl-$(Arg)_3$-OMe was added to GLs consisting only of DOPC, no fluorescence was observed (Figure 5A). Strong fluorescence of dansyl-$(Arg)_3$-OMe was observed on the surface of DOPC GLs containing GM3, GM2, or GM1, depending on the ganglioside contents (Figures 5B−D and 6A). The homogeneous fluorescent images on GL indicate that phase separation of gangliosides was not induced in the DOPC membrane. By contrast, GLs containing zwitterionic SM hardly showed fluorescence by the addition of dansyl-$(Arg)_3$-OMe even at 40% contents (Figure 5G). GLs containing LacCer, which is a sialic acid-deficient structure from GM3, also showed slight fluorescence regardless of its content (Figure 5H). The fluorescence intensity on GLs containing other anionic lipids, sulfatide or DOPG, gradually increased as lipid contents increased (Figure 5E,F), but these intensities were lower than those of monosialyl gangliosides at the same content amounts. These results indicate that dansyl-$(Arg)_3$-OMe recognized monosialyl gangliosides on GLs of DOPC to work as turn-on fluorescent probes.

Figure 6B shows a box plot of the fluorescence intensity distribution of dansyl-$(Arg)_3$-OMe on GLs containing 40% various lipids (N = 20 each), in which P-values were calculated using a t-statistical test compared with data of GM3-containing GLs. The broad distribution of fluorescence intensity may be due to variations in size, morphology, and composition of GLs. The fluorescence intensity distribution on sulfatide, DOPG, SM, and LacCer-containing GLs showed a significant difference compared with GM3-containing GLs ($P < 0.01$). Alternatively, no clear significant difference was found among monosialyl ganglioside-containing GLs because P-values of GM2 and GM1 are 0.0882 and 0.284 compared with that of GM3, respectively. However, the fluorescence titration experiment showed that dansyl-$(Arg)_3$-OMe bound to DOPG, similarly as monosialyl gangliosides (Figure 2B, Table 1), and the binding ability of the probe on DOPG-containing GLs was reduced compared with that on monosialyl ganglioside-containing GLs. It might be due to the difference in local microenvironments such as molecular packing, curvature, and fluidity of lipid assembly. Probably, since DOPG is tightly packed on the GL of DOPC, it might be difficult for the probe to bind the phosphate anion of DOPG on GL.

CONCLUSION

We provided the first proof-of-concept that dansyl-(Arg)3-OMe is a promising fluorescent probe for turn-on sensing and imaging of monosialyl ganglioside on a liposome membrane. Fluorescence titration showed that dansyl-$(Arg)_3$-OMe bound to monosialyl gangliosides and other anionic lipids with association constants on the order of 10^5 M^{-1}, which accordingly enhanced from 6-fold to 7-fold the fluorescence intensity. The probe minimally increased the fluorescence intensity against anionic polysaccharides, indicating that both anionic function and hydrophobic moiety are essential for

https://doi.org/10.1021/acsorginorgau.1c00013
ACS Org. Inorg. Au 2021, 1, 60−67

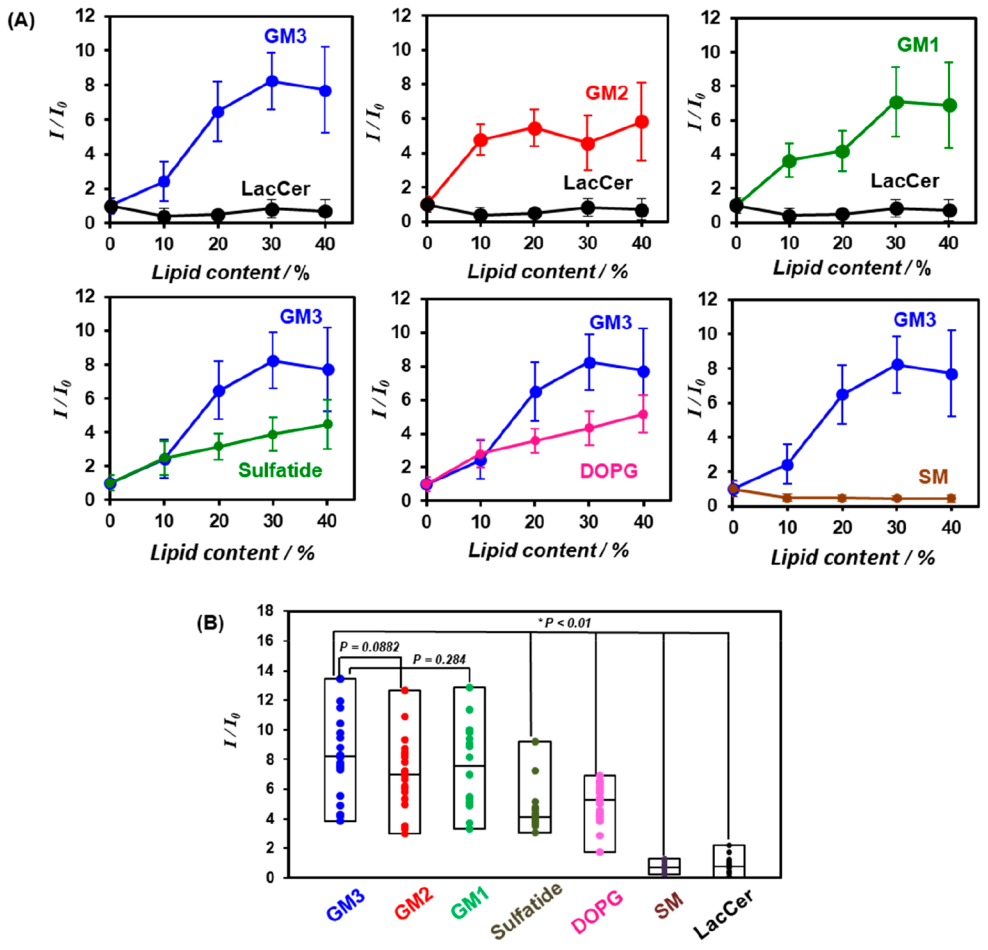


Figure 6. (A) Dependence of the lipid contents in DOPC GLs (N = 20 each) on the normalized fluorescence intensity of dansyl-$(Arg)_3$-OMe obtained by CLSM images in 10 mM HEPES buffer (pH 7.0) at 25 °C. (B) Box plot of the fluorescence intensity distribution of 10 μM dansyl-$(Arg)_3$-OMe on DOPC GLs (N = 20 each) containing 40% monosialyl gangliosides, sulfatide, DOPG, SM, or LacCer.

strong binding and enhancement of fluorescence intensity. The CLSM images revealed that the probe selectively enhanced the fluorescence intensity on GLs containing monosialyl gangliosides compared with the other anionic lipids. Although poor selectivity among monosialyl gangliosides is an issue to be solved soon, we expect that a proper design of the peptide sequence of the probe provides high selectivity among gangliosides. In addition, selective imaging for disialyl and trisialyl gangliosides which are biologically important and abundant in cell membranes is an issue that should be challenged in the future.

This simple molecular design for fluorescence imaging of ganglioside would provide a novel molecular tool for chemical biology. Although the probe moderately turned on the fluorescence on DOPG-containing GLs, the content of DOPG on the cell membrane of animal tissue is low.[45] The gangliosides content used in this study (10−40%) is higher than that on natural cell membranes,[1] but the local concentration of gangliosides in lipid rafts should be higher than the average. We expect that dansyl-$(Arg)_3$-OMe works as a turn-on imaging probe selective to locally enriched gangliosides in lipid rafts on living animal cell membranes. Although the cell penetration ability of oligo-arginine peptides[46] should be considered, the application of the probe for turn-on imaging on cell membranes is currently in progress.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsorginorgau.1c00013.

All experimental details including synthesis of fluorescent probes, estimation of binding affinity, and binding analysis to monosialyl gangliosides on GLs; details of changes of fluorescence spectra; DLS data; and spectroscopic data for probes (PDF)

■ AUTHOR INFORMATION

Corresponding Author

Kazunori Matsuura − *Department of Chemistry and Biotechnology, Graduate School of Engineering and Centre for Research on Green Sustainable Chemistry, Tottori University, Tottori 680-8552, Japan;* orcid.org/0000-0001-5472-7860; Email: ma2ra-k@tottori-u.ac.jp

Authors

Koichi Hisamoto − *Department of Chemistry and Biotechnology, Graduate School of Engineering, Tottori University, Tottori 680-8552, Japan*

https://doi.org/10.1021/acsorginorgau.1c00013
ACS Org. Inorg. Au 2021, 1, 60−67

Tomoya Tanaka − *Department of Chemistry and Biotechnology, Graduate School of Engineering, Tottori University, Tottori 680-8552, Japan*
Ryota Sakamoto − *Department of Chemistry and Biotechnology, Graduate School of Engineering, Tottori University, Tottori 680-8552, Japan*
Mizuki Okazaki − *Department of Chemistry and Biotechnology, Graduate School of Engineering, Tottori University, Tottori 680-8552, Japan*
Hiroshi Inaba − *Department of Chemistry and Biotechnology, Graduate School of Engineering and Centre for Research on Green Sustainable Chemistry, Tottori University, Tottori 680-8552, Japan;* orcid.org/0000-0002-7658-7827

Complete contact information is available at: https://pubs.acs.org/10.1021/acsorginorgau.1c00013

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was supported by Grant-in-Aid for Transformative Research Areas "Molecular Cybernetics" (20A40) from the Ministry of Education (Grant 20H05970). The authors would like to thank MARUZEN-YUSHODO Co., Ltd., for the English language editing.

■ REFERENCES

(1) Sonnino, S.; Mauri, L.; Chigorno, V.; Prinetti, A. Gangliosides as Components of Lipid Membrane Domains. *Glycobiology* **2007**, *17*, 1R−13R.
(2) Lopez, P. H. H.; Schnaar, R. L. Gangliosides in Cell Recognition and Membrane Protein Regulation. *Curr. Opin. Struct. Biol.* **2009**, *19*, 549−557.
(3) Groux-Degroote, S.; Guérardel, Y.; Delannoy, P. Gangliosides: Structures, Biosynthesis, Analysis, and Roles in Cancer. *ChemBioChem* **2017**, *18*, 1146−1154.
(4) Schnaar, R. L. Gangliosides of the Vertebrate Nervous System. *J. Mol. Biol.* **2016**, *428*, 3325−3336.
(5) Maginnis, M. S. Virus−Receptor Interactions: The Key to Cellular Invasion. *J. Mol. Biol.* **2018**, *430*, 2590−2611.
(6) Zhang, X.; Nie, H.; Whited, J.; Wang, D.; Li, Y.; Sun, X.-L. Recent Approaches for Directly Profiling Cell Surface Sialoform. *Glycobiology* **2018**, *28*, 910−924.
(7) Massaad, C. A.; Zhang, G.; Pillai, L.; Azhdarinia, A.; Liu, W.; Sheikh, K. A. Fluorescently-tagged Anti-ganglioside Antibody Selectively Identifies Peripheral Nerve in Living Animals. *Sci. Rep.* **2015**, *5*, 15766.
(8) Whited, J.; Zhang, X.; Nie, H.; Wang, D.; Li, Y.; Sun, X.-L. Recent Chemical Biology Approaches for Profiling Cell Surface Sialylation Status. *ACS Chem. Biol.* **2018**, *13*, 2364−2374.
(9) Badr, H. A.; Elsayed, A. I.; Ahmed, H.; Dwek, M. V.; Li, C.-z.; Djansugurova, L. B. Preferential Lectin Binding of Cancer Cells upon Sialic Acid Treatment Under Nutrient Deprivation. *Appl. Biochem. Biotechnol.* **2013**, *171*, 963−974.
(10) De Chatterjee, A.; Mendez, T. L.; Roychowdhury, S.; Das, S. The Assembly of GM1 Glycolipid- and Cholesterol-Enriched Raft-Like Membrane Microdomains Is Important for Giardial Encystation. *Infect. Immun.* **2015**, *83*, 2030−2042.
(11) Komura, N.; Suzuki, K. G. N.; Ando, H.; Konishi, M.; Koikeda, M.; Imamura, A.; Chadda, R.; Fujiwara, T. K.; Tsuboi, H.; Sheng, R.; Cho, W.; Furukawa, K.; Furukawa, K.; Yamauchi, Y.; Ishida, H.; Kusumi, A.; Kiso, M. Raft-based interactions of gangliosides with a GPI-anchored receptor. *Nat. Chem. Biol.* **2016**, *12*, 402−410.
(12) Suzuki, K. G. N.; Ando, H.; Komura, N.; Fujiwara, T. K.; Kiso, M.; Kusumi, A. Development of New Ganglioside Probes and Unraveling of Raft Domain Structure by Single-molecule Imaging. *Biochim. Biophys. Acta, Gen. Subj.* **2017**, *1861*, 2494−2506.
(13) Davis, A. P. Synthetic Lectins. *Org. Biomol. Chem.* **2009**, *7*, 3629−3638.
(14) Mazik, M. Recent Developments in the Molecular Recognition of Carbohydrates by Artificial Receptors. *RSC Adv.* **2012**, *2*, 2630−2642.
(15) Arnaud, J.; Audfray, A.; Imberty, A. Binding Sugars: From Natural Lectins to Synthetic Receptors and Engineered Neolectins. *Chem. Soc. Rev.* **2013**, *42*, 4798−4813.
(16) Sun, X.; James, T. D. Glucose Sensing in Supramolecular Chemistry. *Chem. Rev.* **2015**, *115*, 8001−8037.
(17) Miron, C. E.; Petitjean, A. Sugar Recognition: Designing Artificial Receptors for Applications in Biological Diagnostics and Imaging. *ChemBioChem* **2015**, *16*, 365−379.
(18) Mazik, M.; Cavga, H. Molecular Recognition of N-Acetylneuraminic Acid with Acyclic Benzimidazolium- and Aminopyridine/guanidinium-Based Receptors. *J. Org. Chem.* **2007**, *72*, 831−838.
(19) Geffert, C.; Kuschel, M.; Mazik, M. Molecular Recognition of N-Acetylneuraminic Acid by Acyclic Pyridinium- and Quinolinium-Based Receptors in Aqueous Media: Recognition through Combination of Cationic and Neutral Recognition Sites. *J. Org. Chem.* **2013**, *78*, 292−300.
(20) Carter, T. S.; Mooibroek, T. J.; Stewart, P. F. N.; Crump, M. P.; Galan, M. C.; Davis, A. P. Platform Synthetic Lectins for Divalent Carbohydrate Recognition in Water. *Angew. Chem., Int. Ed.* **2016**, *55*, 9311−9315.
(21) Francesconi, O.; Milanesi, F.; Nativi, C.; Roelens, S. A Simple Biomimetic Receptor Selectively Recognizing the $GlcNAc_2$ Disaccharide in Water. *Angew. Chem., Int. Ed.* **2021**, *60*, 11168−11172.
(22) Matsubara, T.; Ishikawa, D.; Taki, T.; Okahata, Y.; Sato, T. Selection of Ganglioside GM1-Binding Peptides by Using a Phage Library. *FEBS Lett.* **1999**, *456*, 253−256.
(23) Matsubara, T.; Iijima, K.; Nakamura, M.; Taki, T.; Okahata, Y.; Sato, T. Specific Binding of GM1-Binding Peptides to High-Density GM1 in Lipid Membranes. *Langmuir* **2007**, *23*, 708−714.
(24) Fujitani, N.; Shimizu, H.; Matsubara, T.; Ohta, T.; Komata, Y.; Miura, N.; Sato, T.; Nishimura, S.-I. Structural Transition Study of a Fifteen Amino Acid Residue Peptide Induced by GM1. *Carbohydr. Res.* **2007**, *342*, 1895−1903.
(25) Matsubara, T.; Iida, M.; Tsumuraya, T.; Fujii, I.; Sato, T. Selection of a Carbohydrate-Binding Domain with a Helix-Loop-Helix Structure. *Biochemistry* **2008**, *47*, 6745−6751.
(26) Matsubara, T.; Sumi, M.; Kubota, H.; Taki, T.; Okahata, Y.; Sato, T. Inhibition of Influenza Virus Infections by Sialylgalactose-Binding Peptides Selected from a Phage Library. *J. Med. Chem.* **2009**, *52*, 4247−4256.
(27) Matsubara, T.; IIjima, K.; Kojima, T.; Hirai, M.; Miyamoto, E.; Sato, T. Heterogeneous Ganglioside-Enriched Nanoclusters with Different Densities in Membrane Rafts Detected by a Peptidyl Molecular Probe. *Langmuir* **2021**, *37*, 646−654.
(28) Deshayes, S.; Cabral, H.; Ishii, T.; Miura, Y.; Kobayashi, S.; Yamashita, T.; Matsumoto, A.; Miyahara, Y.; Nishiyama, N.; Kataoka, K. Phenylboronic Acid-Installed Polymeric Micelles for Targeting Sialylated Epitopes in Solid Tumors. *J. Am. Chem. Soc.* **2013**, *135*, 15501−15507.
(29) Matsumoto, A.; Stephenson-Brown, A. J.; Khan, T.; Miyazawa, T.; Cabral, H.; Kataoka, K.; Miyahara, Y. Heterocyclic Boronic Acids Display Sialic Acid Selective Binding in a Hypoxic Tumor Relevant Acidic Environment. *Chem. Sci.* **2017**, *8*, 6165−6170.
(30) Liu, A.; Peng, S.; Soo, J. C.; Kuang, M.; Chen, P.; Duan, H. Quantum Dots with Phenylboronic Acid Tags for Specific Labeling of Sialic Acids on Living Cells. *Anal. Chem.* **2011**, *83*, 1124−1130.
(31) Chaudhary, P. M.; Murthy, R. V.; Yadav, R.; Kikkeri, R. A Rationally Designed Peptidomimetic Biosensor for Sialic Acid on Cell Surfaces. *Chem. Commun.* **2015**, *51*, 8112−8115.
(32) Peng, N.; Xu, R.; Si, M.; Victorious, A.; Ha, E.; Chang, C.; Xu, X. Fluorescent Probe with Aggregation-induced Emission Character-

istics for Targeted Labelling and Imaging of Cancer Cells. *RSC Adv.* **2017**, *7*, 11282−11285.
(33) Strakova, K.; Soleimanpour, S.; Diez-Castellnou, M.; Sakai, N.; Matile, S. Ganglioside-Selective Mechanosensitive Fluorescent Membrane Probes. *Helv. Chim. Acta* **2018**, *101*, e1800019.
(34) Crich, S. G.; Alberti, D.; Szabo, I.; Aime, S.; Djanashvili, K. MRI Visualization of Melanoma Cells by Targeting Overexpressed Sialic Acid with a Gd^{III}-dota-en-pba Imaging Reporter. *Angew. Chem., Int. Ed.* **2013**, *52*, 1161−1164.
(35) Di, H.; Liu, H.; Li, M.; Li, J.; Liu, D. High-Precision Profiling of Sialic Acid Expression in Cancer Cells and Tissues Using Background-Free Surface-Enhanced Raman Scattering Tags. *Anal. Chem.* **2017**, *89*, 5874−5881.
(36) Shinde, S.; El-Schich, Z.; Malakpour, A.; Wan, W.; Dizeyi, N.; Mohammadi, R.; Rurack, K.; Wingren, A. G.; Sellergren, B. Sialic Acid-Imprinted Fluorescent Core−Shell Particles for Selective Labeling of Cell Surface Glycans. *J. Am. Chem. Soc.* **2015**, *137*, 13908−13912.
(37) Klymchenko, A. S. Solvatochromic and Fluorogenic Dyes as Environment-Sensitive Probes: Design and Biological Applications. *Acc. Chem. Res.* **2017**, *50*, 366−375.
(38) Shvadchak, V. V.; Kucherak, O.; Afitska, K.; Dziuba, D.; Yushchenko, D. A. Environmentally Sensitive Probes for Monitoring Protein-Membrane Interactions at Nanomolar Concentrations. *Biochim. Biophys. Acta, Biomembr.* **2017**, *1859*, 852−859.
(39) Zhuang, Y.-D.; Chiang, P.-Y.; Wang, C.-W.; Tan, K.-T. Environment-Sensitive Fluorescent Turn-On Probes Targeting Hydrophobic Ligand-Binding Domains for Selective Protein Detection. *Angew. Chem., Int. Ed.* **2013**, *52*, 8124−8128.
(40) Blondeau, P.; Segura, M.; Pérez-Fernández, R.; de Mendoza, J. Molecular Recognition of Oxoanions Based on Guanidinium Receptors. *Chem. Soc. Rev.* **2007**, *36*, 198−210.
(41) Kataev, E. A.; Müller, C.; Kolesnikov, G. V.; Khrustalev, V. N. Guanidinium-Based Artificial Receptors for Binding Orthophosphate in Aqueous Solution. *Eur. J. Org. Chem.* **2014**, *2014*, 2747−2753.
(42) Schmuck, C. How to Improve Guanidinium Cations for Oxoanion Binding in Aqueous Solution? The Design of Artificial Peptide Receptors. *Coord. Chem. Rev.* **2006**, *250*, 3053−3067.
(43) Onda, M.; Yoshihara, K.; Koyano, H.; Ariga, K.; Kunitake, T. *J. Am. Chem. Soc.* **1996**, *118*, 8524−8530.
(44) Tsumoto, K.; Matsuo, H.; Tomita, M.; Yoshimura, T. Efficient Formation of Giant Liposomes through the Gentle Hydration of Phosphatidylcholine Films Doped with Sugar. *Colloids Surf., B* **2009**, *68*, 98−105.
(45) Uphoff, A.; Hermansson, M.; Haimi, P.; Somerharju, P. Analysis of Complex Lipidomes. In *Medical Applications of Mass Spectrometrys*; Elsevier: Amsterdam, 2008; Chapter 11, pp 223−249.
(46) Futaki, S.; Nakase, I. Cell-Surface Interactions on Arginine-Rich Cell-Penetrating Peptides Allow for Multiplex Modes of Internalization. *Acc. Chem. Res.* **2017**, *50*, 2449−2456.

https://doi.org/10.1021/acsorginorgau.1c00013
ACS Org. Inorg. Au 2021, 1, 60−67