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PAPER

A hydrophobic disordered peptide spontaneously anchors a covalently bound RNA hairpin to giant lipidic vesicles

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The attraction of nucleic acids to lipidic compartments is the first step for carriers of potentially inheritable information to self-organise in functionalised synthetic cells. Confocal fluorescence imaging shows that a synthetic amphiphilic peptidyl RNA molecule spontaneously accumulates at the outer bilayer membranes of phospho- and glycolipidic giant vesicles. Cooperatively attractive interactions of –3.4 to –4.0 kcal/mol between a random coil hydrophobic peptide and lipid membranes can thus pilot lipophobic RNA to its compartmentation. The separation of mixed lipid phases in the membranes further enhances the local concentration of anchored RNA.

Introduction

The construction of synthetic living cells from a minimum of predisposed genetic information — ideally none at all — is a declared goal of a growing community of chemists.[1] Natural liposomes are compartments ideal for the growth and harboring of information carriers,[2] useful metabolites and for their own reproduction.[3] The bilayer surface of lipidic vesicles may have served since long for the anchoring and enrichment of macromolecules which sooner or later have been internalised and retained in the interior of the vesicles.[2,4] An obvious challenge for the deliberate creation of dynamic off-equilibrium systems that are expected to show 'signs of life' — i.e. sustained population growth of certain, not all initially present individuals — is the concentration and compartmentation of nucleic acids at and into lipidic vesicles.[5] This can be achieved in two ways ; initially premixed and dried lipids and nucleic acids, or their precursors, may become trapped within vesicles as they form through hydration,[6] or else the nucleic acids are compartmented through the use of helper compounds such as artificial cationic lipids and/or lipidic anchors, including fatty acid glycerol esters, terpenes, tocopherol and cholesterol.[7]

Here for the first time we find that a hydrophobic peptide serves as an efficient anchoring device for lipophobic RNA. The importance of utilising peptides rather than lipidic compounds as anchors is that they are easier to evolve and to explore through in vitro selection protocols. Just how well the lipophilicity of a peptide compensates for the hydrophilicity of RNA is an unexpected outcome of this study.

This first model system investigated the interaction between an amphiphilic informational chimeric molecule, obtained from joining a synthetic hydrophobic peptide with synthetic RNA, and giant vesicles (GVs) composed of natural phospholipids and synthetic glycolipids. The main goal was to see whether an amphiphilic peptide-RNA conjugate, composed of a polyanionic RNA 'head' and a hydrophobic peptide 'tail' of comparable size, would stably interact with vesicles of a variety of lipid compositions, spontaneously enrich at the water-lipid interface, perhaps get across the membrane and eventually become encapsulated.

We used glycolipids in some of the GVs due to their potential protective effect under dehydration conditions and for their phase patterning properties in lipid membranes.[8] Spontaneous or glycan-induced demixing of fluid phases of lipid membranes has an important bearing on the formation of lipid rafts which, in evolved cellular systems, are involved in the spatial organisation of membrane-anchored proteins.[9] One might therefore expect lipid-anchored peptidyl RNA to enrich and concentrate even more at phase-separated fluid vesicle surfaces.

The peptide sequence of the peptidyl RNA that we chose to begin with (Fig. 1) was a lipophilic 20-mer containing six leucines (L) and fourteen alanines (A). The predicted properties of the related model icosapeptide amide H_3N^+–$LA_3LA_2LA_3LA_2LA_3LA$–CO-NH_2 at pH 7.0, T = 298 K, and I = 0.15 M (salt) were propensities of 63, ≤ 67 and ≤ 86 % for an α-helix, coiled-coils, and β-sheet aggregations, respectively. According to the empirical Wimley & White peptide hydrophobicity scale,[10] $\Delta G°$ (25 °C) = –3.15 kcal mol^{-1} lipid-water partitioning in favour of the lipid membranes

was calculated for this icosapeptide amide by *Membrane Protein Explorer* (MPEx).

Experimental Design, Results and Discussion

Peptidyl RNA, an informational amphiphile. Our icosamer H_3N^+–$LA_3LA_2LA_3LA_2LA_3LA$-3'-amino-3'-deoxyadenosine (peptidyl-NHdA), bearing a cationic N-terminus and being C-terminally linked through an amide bond to adenosine (Fig. 1b), showed circular dichroism (CD) spectra at both 25 and 4 °C being dominated by a single strong negative ellipticity below 200 nanometers wavelength (Fig. S21).† No signature of an α-helix was found. The infrared (IR) analysis of the peptide's amide I region confirmed that the icosamer's conformation was 84–88 % disordered and 12–16 % β-sheeted (Fig. S25),† rising to 22 % β in the presence of 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphatidylcholine (POPC) at a molar lipid-to-peptide (L/P) ratio 40:1 (Table S5).† Peptidyl-NHdA was probably too short to persistently favor any defined ordered conformation.[11]

Fig. 1 Peptidyl-RNA(FAM) = Amphiphilic (**a**), hydrolytically 3'-stabilised (**b**) peptidyl RNA labeled with fluorescein (**c**). RNA C*UUCG*G tetraloop NMR structure (**d**), and Ala_{20} peptide models 3'-connected to RNA hairpin NMR structure (**e**).

The RNA part of the peptidyl RNA was a 22-meric hairpin stem-loop molecule that was mimicking the aminoacyl stem of transfer RNA (Fig. 1a). In the present work the connection between peptide and RNA was a hydrolytically stable amide bond being isosteric with the natural but hydrolytically labile ester function usually found in peptidyl transfer RNA. This replacement of oxygen with nitrogen at the 3'-terminal position (Fig. 1b) was needed to reliably observe the constructs in clear fluorescence images over a few hours, up to several days. The stem-loop folding of the RNA hairpin, closed by UUCG, was previously shown to be fully stable at least up to 60 °C (T_m = 87.6 °C).[12] Based on this RNA hairpin's NMR solution structure (model 4 of Protein Data Bank code 1IKD),[13] where the unpaired cytosine of the tetraloop UUCG appeared unstacked (as opposed to the UUCG tetraloops in X-Ray structure 1F7Y)[14] from the uracil of the neighboring non-canonical G_{syn}·*U* pair (Fig. 1d), the peptidyl RNA was made specifically visible through the replacement of the loop cytidine residue by a thymidine residue lacking 2'-OH (shown in brackets in Fig. 1d) and labelled with the green-fluorescent 6-fluoresceinyl carboxamido (FAM) fluorophore (Fig. 1c). The vertical green arrow in Fig. 1d points at C5, the carbon atom to which the FAM spacer was attached to the RNA loop.

Highly amphiphilic peptidyl RNAs, composed of a large polar 'head' (the RNA hairpin) and a single lipophilic 'tail' (the peptide), can be considered as particularly large detergent molecules (Fig. 1e). The RNA head of this conjugate was about 5.0 nm long × 2.0-2.2 nm wide, which is typical for RNA in an A conformation. Depending on the peptide's conformation, the peptide tail could be 6.7-2.8 nm long × 0.60-0.85 nm wide, resulting in a head-to-tail width ratio 3.3-3.6 for a β–strand and 2.3-2.5 for an α–helix. The truncated cone dimension, as expressed by the head/tail width ratio, of the peptidyl RNA bearing a disordered 'random coil' peptide is somewhere in between these extreme values, width ratio approximately 2.5-3.3. Such conjugates do form micelles in water at very low concentrations and, when deposited on a glass surface, they self-aggregate in multilamellar spheroids and can spread into multilamellar supported bilayers.[15]

Lipid vesicles as hosts. GVs are micrometer-sized, thus, membrane interactions on GVs can be directly and conveniently visualised by light microscopy. We considered the 'natural swelling' method, viz. the slow and undisturbed 'gentle' hydration of a dry film of phospho- and glycolipid mixtures, to be most appropriate, particularly, in mimicking the formation conditions of lipidic GVs as potential protocells during early stages of molecular evolution.

The chemical composition of the GVs was varied, in order to form either monophasic disorderly liquid membranes at 25 °C using POPC and 1,2-dioleoyl-*sn*-glycero-3-phosphatidylcholine (DOPC), or biphasic membranes containing admixed higher melting 1,2-dipalmitoyl-*sn*-glycero-3-phosphatidylcholine (DPPC), cholesterol (CHOL), very fluid oleate, 1,2-dipalmitoyl-*sn*-glycero-3-α-D-mannopyranoside (DP-α-Man), or -β-D-glucopyranoside (DP-β-Glc). The lipid membranes were specifically labelled with 0.01–0.2 mol % red-fluorescent 1,2-dioleoyl-*sn*-glycero-3-phosphatidylethanolamine-*N*-lissamine rhodamine B sulfonate (DOPE-Rh). In mixed lipid GVs, which had been prepared in part at elevated temperatures from hydrating dry films of DOPC/DPPC 1:4, DOPC/DPPC/CHOL 1:3:1 or POPC/oleate 85:15, spontaneous lipid demixing into liquid-crystalline and gel phases could be monitored at ambient temperature by imaging DOPE-Rh.[16]

To assemble glycolipidic vesicles DP-α-Man or DP-β-Glc were admixed with POPC prior to hydration. Their insertion and compatibility with POPC bilayers was assessed by imaging giant POPC vesicles that contained 5 mol % turquoise-fluorescent 1,2-dipalmitoyl-*sn*-glycero-3-(6-dansylamino-6-deoxy)-β-D-glucopyranoside, DP-β-(Dns)Glc (Image File S1).† Cohydrated glycolipids were tolerated up to 20 mol % without being visibly detrimental to the shape and stability of the GVs. This was confirmed by dynamic light scattering (DLS) from large unilamellar vesicles (LUVs) prepared from POPC and varying amounts of glycolipid. The LUV's average hydrodynamic radius remained in the range of 50–80 nanometers between 0 and 25 mol % glycolipids (Fig. 2).†

Fig. 2 DLS study on glycolipid-POPC LUVs

Natural swelling produced a large variety of GVs in the 5–30 micrometer diameter range. They were predominantly spherical or spheroidal, multilamellar and multivesicular, time and again of more complex topology (*cf.* collection of exotic examples in Fig. S13.1)† and sometimes but rarely unilamellar (GUVs). GVs composed of POPC presented their membranes as a homogeneous

phase. Heterogeneous zones containing enriched DOPE-Rh in the membranes became clearly visible in the fluorescence images of certain glycolipid-POPC GVs and all mixed-lipid GVs prepared from DOPC/DPPC 1:4 (Fig. S13.2-S13.4).†

GVs : Anchoring, partitioning and impermeability. When a solution of peptidyl-RNA(FAM) was added to a population of GVs at L/P 150, 300 or 1500:1, the green fluorescence immediately appeared enriched at their outer lipid membranes (Fig. 3, upper). No evident differences could be discerned in the images of anchoring experiments on pure POPC GVs when compared to those that contained 15 mol % glycolipids in their membranes. The green rings appeared within the mixing time of a few seconds and persisted for at least 18 hours at ambient temperature. Anchored peptidyl-RNA(FAM) homogeneously covered monophasic GVs (Figs. 3.1–3.4 and S14–S15)†, whereas on biphasic mixed-lipid GVs they spontaneously demixed in highly enriched and rather depleted (green) zones, but merely over the outermost membranes (Fig. 4).

Fig. 3 Upper: Incubation of red lipidic GVs with green peptidyl-RNA(FAM). Fluorescence micrographs after incubation of monophasic GVs. Single confocal 1 micron-thin slices through GVs (**1**-**4**) using separate green and red detection channels.

Fig. 4 Fluorescence micrographs after incubation of red mixed-lipid GVs with green peptidyl-RNA(FAM). Single confocal 1 micron-thin slices through GVs (**1**-**2**) and superimposed cumulation of many confocal slices at all optical depths of the GVs (**3**-**4**); **1**, **2** and **3 right** : red + green superimposed.

Intact naturally swelled GV membranes, as well as GVs that had been incubated with peptidyl-NHdA at L/P 300 beforehand, were impermeable for calcein, neither when added from the outside, nor was encapsulated calcein leaking from the inside of GUVs that had been prepared through emulsion droplet transfer.[17,18] GVs covered with peptidyl-RNA(FAM) remained impermeable for added calcein unable to visibly displace anchored peptidyl-RNA(FAM) from the membranes.† No green rings were found after incubating the GVs with the fluorescent control compound RNA(FAM) peptide exempt but under otherwise identical conditions.†

The experimental free energy of partitioning of the peptidyl RNA was determined from fluorescence micrographs to be $\Delta G°$ = –3.4 to –4.0 kcal mol^{-1} equivalent to K = 300:1 to 780:1 for excess anchored over unanchored peptidyl-RNA(FAM) in the presence of 0.1 mM lipids at 25 °C.† In all images where peptidyl-RNA(FAM) was seen anchored to monophasic GVs, a prevailing population of quite uniform and comparatively low intensity, viz. 'normal' green rings, and a minority of particularly bright green rings were observed in the same experiments.† A careful ring profile analysis revealed that the brighter rings showed a consistently two to three-fold higher intensity when compared to the normal green rings. The distribution of relative peak areas of many bright rings gave evidence for a highly discontinuous intensity distribution of the green ring fluorescence.† A far from normal, highly discontinuous intensity distribution of the anchoring density on the vesicles is consistent with the presence of aggregated sheets of peptidyl RNA being anchored at the outer water-lipid interface of the giant vesicles and cooperatively stabilised by peptide-peptide interactions.

Other intriguing and recurrent observations were : *(i)* Upon gentle hydration of single-lipid or mixed-lipid dry films, an estimated 10-25 % of the observed GVs produced sustained filaments that protruded from the outer membranes (Fig. 3.2-3.3). These filaments were particularly tuned to the anchoring of peptidyl-RNA(FAM) which readily assembled in protruding seemingly helical multilamellar superstructures (Fig. 4.1, 4.3, see also depth-resolved multi-channel analyses in Image Files S3 and S4).† *(ii)* The spontaneous local enrichment of peptidyl-RNA(FAM) being anchored to the spherical outer membranes of mixed-lipid biphasic GVs, as evidenced by highly green-fluorescent zones next to depleted green areas (Fig. 4), is quite remarkable and was never observed on monophasic GVs (Fig. 3).

Taken all together, these observations indicate that the filaments and the covering of the outermost surface of lipidic vesicles were nourished from self-aggregated[15] amphiphilic peptidyl RNA, which preferred the most delicate thin lipidic structures to firstly anchor to. These anchored peptidyl RNA were in minor parts composed of multilayered sheets still stabilised by peptide-peptide interactions. In other words, peptidyl-RNA(FAM) would not completely disintegrate into separate chimeric molecules but remain aggregated upon anchoring to lipidic bilayers, preferentially as peptidyl RNA monolayers in normal rings, or else as double- or triple-layers in bright rings. At very low peptidyl RNA concentrations 'first hit' GVs would thus enrich cooperatively anchored peptidyl RNA molecules at their surface by stripping them off from predisposed peptidyl RNA self-aggregates. Consequently, a majority of the remaining GVs would linger untouched, or become only very weakly covered, resulting in an even more abnormal distribution of green fluorescence intensity density, which is reminiscent of molecular crowding.[18]

It seems as if the green filaments were illustrating the anchoring process: the initial interaction between self-aggregated peptidyl RNA and very thin lipid filaments, and the stripping off of peptidyl RNA layers to cover the outermost lipid bilayer of the vesicles. In such a context, very similar CD and IR spectra of the icosapeptide, irrespective of the absence or presence of a fourty-fold molar excess POPC,† did not come with a surprise. In both self-aggregated and lipid-anchored appearances the intermolecular disordered peptide-peptide interactions prevailed.

Permeability through LUV membranes. In a series of experiments, we tested pyranine-containing LUVs prepared from

POPC for treatments with known amounts of externally added *(i)* peptidyl RNA, *(ii)* the icosameric peptide control (peptidyl-NHdA), *(iii)* control RNA, *(iv)* control solvents (water, methanol, DMSO/ethanol 1:1). After the addition of each of these "agents" (solvent or compound) and a short incubation at room temperature (3 min), 0.1 N HCl was added. Caused by the acidification of the LUV's cores, the pyranine fluorescence revealed the actual intra-liposomal pH drop.[19] This value was compared with the '100 % permeation' measured after Triton X-100 addition.† The addition of the control solvents induced a pH drop that on average was about 30–40 % with respect to the total LUV's permeabilisation (*cf.* entries 1 and 2, Table 1). We ascribed these changes to free (released) pyranine present in the sample and therefore considered a 'net proton permeability increase', being specific for the LUV's interaction with a macromolecular compound, only above this background (5th column of Table 1).

The treatment of LUVs with peptidyl RNA at L/P 2100:1 gave a response for proton transport similar to that of gramicidin A at L/P 214:1 (Table 1, entries 2 and 3). This is in sharp contrast with the microscopically observed impermeability for calcein through GV membranes to which peptidyl-RNA(FAM) had anchored at L/P 300:1. It indicates that bilayer-anchored lipophilic peptides formed molecular architectures that allowed for the binding and diffusion of small cations, such as hydronium ions (hydrated protons) and possibly sodium or potassium cations (*cf.* MALDI ToF spectra in Figure S8 and Figure Legend S8),† but not for molecules of the size of calcein, as shown by control experiments. A difference between the permeability of membranes of GVs and LUVs, owing to a much higher curvature and thus membrane strain in the latter, cannot be excluded. Interestingly, the control peptide 'peptidyl-NHdA' promoted a pronounced and dose-dependent acidification inside the POPC LUVs, whereas the addition of comparable amounts of added RNA did not (entries 4 and 5).

In short, the incubation of POPC LUVs with peptidyl-NHdA at L/P 200:1 gave no measurable rise to changes in neither size distribution nor turbidity of the LUV suspensions.† The incubation of pyranine-encapsulated LUVs with RNA(FAM) had no significant effect above the negative controls. In contrast, peptidyl RNA and peptidyl-NHdA caused a prominent increase in proton permeation comparable to the one of pore-forming gramicidin A. The proton transport-enhancing effect of the peptide was clearly dose-dependent. Hence, the permeability assays showed that peptidyl RNA anchoring was not detrimental to membrane stability, as evidenced by the failure of evident calcein permeation. The observation on proton permeability suggested a locally specific lipid-peptide architecture that could have functioned as a passive carrier of hydronium ions, perhaps alkali metal ions.

Table 1. Agent-dependent LUV permeability for protons.

Entry	Tested agent	Molar lipid-to-agent ratio	Relative pH drop[c] [%] (±5 %)	Net proton permeability increase[d] [%]
1	water methanol DMSO/EtOH (1:1)	Same volumes as in entries 2–5	35	0
2	Gramicidin A (in DMSO/EtOH 1:1)	214:1	100	+65
3	peptidyl RNA (in water)	2100:1	97	+62
4	peptidyl-NHdA (in methanol)	900:1 300:1 150:1	50 87 100	+15 +52 +65
5	RNA (in water)	210:1	44	+9

c Absolute pH as determined from $\log[F_{460}/F_{406}]$ (Figure S19c).† Relative pH drop : 0 % = no treatment; 100 % = after addition of Triton X-100.

d = relative pH drop[c] – 35 % (background from entry 1).

Conclusion

An amphiphilic icosanionic peptidyl RNA bearing a hydrophobic, apart from the amino terminus charge-neutral Leu_6Ala_{14} peptide, spontaneously anchored to and enriched at the accessible bilayer membranes of giant vesicles and stayed there. This designed peptide was destitute of any particularly exceptional transmembrane association properties or pronounced folding characteristics, and it interacted with the lipids as if undisturbed by the RNA. The anchoring to GV membranes did not disrupt the strong inter-peptide interactions already present in the conjugate's self-aggregates. It did not provoke any crossing of the conjugate, or of calcein, through the intact bilayer membranes. The precise nature of peptide-lipid interactions remains to be solved. Reversibly conjugated peptide-RNA ester libraries of limited average half-lives shall be used in the future, to find conjugates with membrane-crossing properties that permit their spontaneous encapsulation by lipidic GVs.[29]

Experimental

Synthesis of DP-β-Glc

1,2-Di-*O*-benzyl-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-*sn*-glycerol. Boron trifluoride etherate (10 μL) was added at –15 °C to a cold solution of 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl trichloroacetimidate[20] (1.97 g, 4.00 mmol) and 1,2-di-*O*-benzyl-*sn*-glycerol[21] (0.750 g, 2.75 mmol) in dry CH_2Cl_2 (10 mL) containing activated 4Å molecular sieves. The mixture was stirred for 2 h at –15 °C and allowed to warm to 0 °C and stirred 2 h more. After addition of CH_2Cl_2 (60 mL) and filtration, the organic phase was washed with staturated $NaHCO_3$ solution (10 mL) and dried (Na_2SO_4). The organic phase was concentrated under reduced pressure, the crude product was purified by silica-gel

column chromatography (3:4 ethyl acetate-PE) to give pure target compound[22] as an oily material. 1.31 g, 79% yield. R_f 0.50; $[\alpha]_D^{20}$ –12.5 (*c* 1.0, $CHCl_3$) [lit[23] $[\alpha]_D^{20}$ –12.5 (*c* 1.0, $CHCl_3$)]; ^{1}H NMR ($CDCl_3$): δ 7.38-7.24 (m, 10H, $2C_6H_5$), 5.20 (dd, 1H, $J_{2,3}$ 9.4, $J_{3,4}$ 9.4 Hz, H-3), 5.09 (dd, 1H, $J_{4,5}$ 9.7 Hz, H-4), 5.02 (dd, 1H, $J_{1,2}$ 8.0 Hz, H-2), 4.68 and 4.63 (AB system, *J* 12.3 Hz, CH_2Ph), 4.58 and 4.51 (AB system, 2H, *J* 12.4 Hz, CH_2Ph), 4.55 (d, 1H, H-1), 4.26 (dd, 1H, $J_{5,6a}$ 4.7, $J_{6a,6b}$ 12.3 Hz, H-6a), 4.11 (dd, 1H, $J_{5,6b}$ 1.9 Hz, H-6b), 4.00-3.94 (m, 1H, H-$3a_{gly}$), 3.79-3.70 (m, 2H, H-2_{gly}, H-$3b_{gly}$), 3.65 (ddd, 1H, H-5), 3.59 (d, 2H, *J* 3.9 Hz, H-$1a_{gly}$, H-$1b_{gly}$), 2.06, 2.02, 2.00, 1.95 (4s, 12H, $4CH_3COO$): ^{13}C NMR ($CDCl_3$): δ 170.60, 170.20, 169.38, 168.98 (CH_3COO), 138.49, 138.20, 128.41-127.62 (C_6H_5), 101.02 (C-1), 76.82 (C-2_{gly}), 73.43, 72.09 (CH_2Ph), 72.79 (C-3), 71.76 (C-5), 71.31 (C-2), 69.69 (C-1_{gly}), 69.21 (C-3_{gly}), 68.39 (C-4), 61.89 (C-6), 20.70, 20.57, 20.57, 20.57 (CH_3COO). HRMS (ESI) calc. for $C_{31}H_{38}NaO_{12}$, 625.2255; found 625.2265.

3-*O*-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)-1,2-*O*-isopropylidene-*sn*-glycerol. 1,2-Di-*O*-benzyl-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-*sn*-glycerol (0.602 g, 1.00 mmol) and 10 % Pd/C (50 mg) in a 2:1 CH_2Cl_2-EtOH mixture (15 mL) were stirred overnight under hydrogen pressure (6 atm). After filtration through celite and concentration, the crude product was dissolved in acetone (5 mL); 2,2-dimethoxypropane (0.7 mL) and *p*-TsOH (10 mg) were added and the mixture was stirred for 4 h. The solution was neutralised with NEt_3 (20 μL), concentrated under reduced pressure and the crude product was purified by silica gel column chromatography (1:1 EtOAc-PE). The target compound was obtained as a solid. 0.355 g, 77% yield. R_f 0.65, mp 109-110°C (lit[24] mp 117-119°C), $[\alpha]_D^{20}$ –11.5 (*c* 1.0, $CHCl_3$) [lit[20] $[\alpha]_D^{20}$ –11.3 (*c* 1.0, $CHCl_3$)]; ^{1}H NMR ($CDCl_3$): δ 5.10 (dd, 1H, $J_{2,3}$ 9.5, $J_{3,4}$ 9.4 Hz, H-3), 4.97 (dd, 1H, $J_{4,5}$ 9.9 Hz, H-4), 4.89 (dd, 1H, $J_{1,2}$ 8.0 Hz, H-2), 4.53 (d, 1H, H-1), 4.17 (dd, 1H, $J_{5,6a}$ 4.9, $J_{6a,6b}$ 12.3 Hz, H-6a), 4.17-4.12 (m, 1H, H-2_{gly}), 4.03 (dd, 1H, $J_{5,6b}$ 2.3 Hz, H-6b), 3.91 (dd, 1H, $J_{1agly,2gly}$ 6.5, $J_{1agly,1bgly}$ 8.2 Hz, H-$1a_{gly}$), 3.77 (dd, 1H, $J_{2gly,3agly}$ 4.2, $J_{3agly,3bgly}$ 10.7 Hz, H-$3a_{gly}$), 3.69 (dd, 1H, $J_{1bgly,2gly}$ 6.1 Hz, H-$1b_{gly}$), 3.63 (ddd, 1H, H-5), 3.54 (dd, 1H, $J_{2gly,3bgly}$ 5.8 Hz, H-$3b_{gly}$), 1.99, 1.95, 1.92, 1.90 (4s, 12H, $4CH_3COO$), 1.31, 1.24 (2s, 6H, $(CH_3)_2C$); ^{13}C NMR ($CDCl_3$): δ 170.51, 170.12, 169.33, 169.21 (CH_3COO), 109.25 ($C(CH_3)_2$), 100.87 (C-1), 74.23 (C-2_{gly}), 72.72, 71.76, 71.11 (C-2, C-3, C-5), 69.11 (C-3_{gly}), 68.36 (C-4), 66.07 (C-1_{gly}), 61.86 (C-6), 26.51, 25.13 ($C(CH_3)_2$), 20.66, 20.57, 20.52, 20.50 (CH_3COO).

3-*O*-(2,3,4,6-Tetra-*O*-benzyl-β-D-glucopyranosyl)-1,2-*O*-isopropylidene-*sn*-glycerol. 3-*O*-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)-1,2-*O*-isopropylidene-*sn*-glycerol (0.670 g, 1.45 mmol) was stirred overnight in MeOH (25 mL) containing a catalytic amount of MeONa; after concentration and coevaporation from toluene (2x10 mL), the crude residue was dissolved in DMF (10 mL), BnBr (5 equiv.) and Bu_4NI (20 mg) were added and the mixture was cooled to 0 °C under argon. Sodium hydride (6 equiv.) was slowly added at 0 °C and stirring was maintained overnight at rt. The mixture was cooled again, and MeOH was added to destroy the excess of BnBr and NaH. The mixture was poured in icy water, and extracted twice with EtOAc (2x30 mL). The organic phase was washed with water (10 mL), dried and concentrated under reduced pressure. Purification by silica gel column chromatography (1:4 EtOAc-PE) afforded the target compound in 84% yield. Solid; mp 86-87°C, [lit[25] mp 91-91.7°C]; R_f 0.57 (1:3 EtOAc-PE), $[\alpha]_D^{20}$ = +10.6 (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.40-7.17 (m, 20H, $4C_6H_5$), 4.95 and 4.83 (AB system, 2H, *J* 10.8 Hz, CH_2Ph), 4.94 and 4.73 (AB system, 2H, *J* 11.0 Hz, CH_2Ph), 4.85 and 4.56 (AB system, 2H, *J* 10.6 Hz, CH_2Ph), 4.64 and 4.57 (AB system, 2H, *J* 12.2 Hz, CH_2Ph), 4.45 (d, 1H, $J_{1,2}$ 7.8 Hz, H-1), 4.37 (dddd, 1H, $J_{1agly,2gly}$ 6.3, $J_{1bgly,2gly}$ 6.0, $J_{2gly,3agly}$ 5.0, $J_{2gly,3bgly}$ 6.4 Hz, H-2_{gly}), 4.09 (dd, 1H, $J_{1agly,1bgly}$ 8.3 Hz, H-$1a_{gly}$), 4.04 (dd, 1H, $J_{3agly,3bgly}$ 10.2 Hz, H-$3a_{gly}$), 3.87 (dd, 1H, H-$1b_{gly}$), 3.76 (dd, 1H, $J_{5,6a}$ 2.1, $J_{6a,6b}$ 10.8 Hz, H-6a), 3.71 (dd, 1H, $J_{5,6b}$ 1.3 Hz, H-6b), 3.67-3.59 (m, 3H, H-3, H-4, H-$3b_{gly}$), 3.52-3.46 (m, 2H, H-2, H-5), 1.44-1.38 (2s, 6H, $(CH_3)_2C$); ^{13}C NMR ($CDCl_3$): δ 138.72, 138.56, 138.26, 138.22, 128.55-127.27 (C_6H_5), 109.53 ($C(CH_3)_2$), 103.99 (C-1), 84.81 (C-3), 82.29 (C-2), 77.91 (C-4), 75.86, 75.17, 75.01, 74.48 ($CH_2C_6H_5$), 75.05 (C-5), 74.48 (C-2_{gly}), 70.47 (C-3_{gly}), 68.98 (C-6), 67.06 (C-1_{gly}), 27.02, 25.53 ($C(CH_3)_2$).

3-*O*-(2,3,4,6-Tetra-*O*-benzyl-β-D-glucopyranosyl)-*sn*-glycerol. Water (1.0 mL) and TFA (1.0 mL) were successively added to a solution of 3-*O*-(2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranosyl)-1,2-*O*-isopropylidene-*sn*-glycerol (0.650 g, 1.00 mmol) in CH_2Cl_2 (10 mL) and the mixture was stirred for 1 h. After dilution with CH_2Cl_2 (40 mL), the organic phase was washed to neutrality with saturated $NaHCO_3$ solution and the aqueous solution was extracted once with CH_2Cl_2 (10 mL). The combined organic phases were dried (Na_2SO_4) and concentrated. Purification by silica gel column chromatography (3:1 EtOAc-PE) afforded the target compound in 83% yield: 0.475 g. Solid; R_f 0.71: mp 101-102°C [lit[23] mp 101.5-102.4]; $[\alpha]_D^{20}$ +18.1 (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.41-7.13 (m, 20H, $4C_6H_5$), 4.97-4.75 and 4.61-4.51 (m, 8H, $4C_6H_5CH_2$), 4.43 (d, 1H, $J_{1,2}$ 7.8 Hz, H-1), 3.97 (d, 1H, $J_{2gly,3agly}$ 2.4, $J_{3agly,3bgly}$ 11.3 Hz, H-$3a_{gly}$), 3.92-3.86 (m, 1H, H-2_{gly}), 3.77 (dd, 1H, $J_{2gly,3bgly}$ 6.6 Hz, H-$3b_{gly}$), 3.73-3.64 (m, 3H, H-3, H-6a, H-$1a_{gly}$), 3.60 (ddd, 1H, $J_{4,5}$ 9.0, $J_{5,6a}$ 1.8, $J_{5,6b}$ 4.3 Hz, H-5), 3.57-3.53 (m, 3H, H-4, H-6b, H-$1b_{gly}$), 3.48 (dd, 1H, $J_{2,3}$ 9.0 Hz, H-2), 2.16 (t, 1H, *J* 5.9 Hz, O*H*); ^{13}C NMR ($CDCl_3$): δ 138.56, 138.36, 137.97, 137.79, 128.56-127.80 (C_6H_5), 104.42 (C-1), 84.73 (C-3), 82.21 (C-2), 77.92 (C-4), 75.85, 75.15, 75.08, 73.73 ($CH_2C_6H_5$), 73.65 (C-3_{gly}), 74.57 (C-5), 71.27 (C-2_{gly}), 69.00 (C-6), 63.46 (C-1_{gly}). Anal. Calcd for $C_{37}H_{42}O_8$ (614.72): C, 72.29; H, 6.89. Found; C, 71.87; H, 6.93.

3-*O*-(2,3,4,6-Tetra-*O*-benzyl-β-D-glucopyranosyl)-1,2-di-*O*-hexadecanoyl-*sn*-glycerol. A solution of hexadecanoyl chloride (1.25 mmol) in CH_2Cl_2 (2.5 mL) was added dropwise to a solution of 3-*O*-(2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranosyl)-*sn*-glycerol (0.307 g, 0.50 mmol) and pyridine (10.0 mmol, 0.80 mL) in CH_2Cl_2 (2.0 mL). The mixture was stirred overnight, then the excess of hexadecanoyl chloride was destroyed by addition of MeOH at 0°C and stirring was maintained for 2 h. Dichloromethane (30 mL) was added, and the organic phase was successively washed with saturated $NaHCO_3$ solution (10 mL) and H_2O (10 mL).

After concentration, the crude residue was purified by silica gel column chromatography (1:4 EtOAc-PE, then 6:1 CH_2Cl_2-Me_2CO) to give the target compound in 78% yield. Oily material; R_f 0.85 (1:4 EtOAc-PE); $[\alpha]_D^{20}$ +8.6 (c, 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.44-7.15 (m, 20H, 4C$_6H_5$), 5.33 (ddd, 1H, $J_{1agly,2gly}$ 3.7, $J_{1bgly,2gly}$ 6.7, $J_{2gly,3agly}$ 4.5, $J_{2gly,3bgly}$ 4.3 Hz, H-2$_{gly}$), 4.97 and 4.55 (AB system, 2H, J 11.5 Hz, CH_2C$_6$H$_5$), 4.96 and 4.82 (AB system, 2H, J 10.8 Hz, CH_2C$_6$H$_5$), 4.86 and 4.70 (AB system, 2H, J 11.0 Hz, CH_2C$_6$H$_5$), 4.66 and 4.57 (AB system, 2H, J 12.3 Hz, CH_2C$_6$H$_5$), 4.45 (dd, 1H, $J_{1agly,1bgly}$ 11.9 Hz, H-1a$_{gly}$), 4.40 (d, 1H, $J_{1,2}$ 7.5 Hz, H-1), 4.27 (dd, 1H, H-1b$_{gly}$), 4.11 (dd, 1H, $J_{3agly,3bgly}$ 10.8 Hz, H-3a$_{gly}$), 3.80-3.61 (m, 5H, H-3, H-4, H-6a, H-6b, H-3b$_{gly}$), 3.51-3.46 (m, 2H, H-2, H-5), 2.32 (q, 4H, J 6.9 Hz, 2COCH_2), 1.69-1.52 (m, 4H, 2COCH$_2$CH_2), 1.40-1.20 (m, 48H, 24CH_2 alkyl chains), 0.89 (t, 6H, J 6.8 Hz, 2CH_3 alkyl chains); ^{13}C NMR ($CDCl_3$): δ 173.49, 173.15 (C$_{15}$H$_{31}$*C*OO), 138.72, 138.52, 138.23, 128.52-127.72 (*C*$_6$H$_5$), 104.00 (C-1), 84.70 (C-3), 82.10 (C-2), 77.80 (C-4), 75.81, 75.16, 74.89, 73.64, (*C*H$_2$C$_6$H$_5$), 75.08 (C-5), 70.15 (C-2$_{gly}$), 68.88 (C-6), 68.06 (C-3$_{gly}$), 62.83 (C-1$_{gly}$), 34.38, 34.22, 32.05, 29.81-29.21, 25.00, 22.80 (*C*H$_2$ alkyl chains), 14.24 (*C*H$_3$ alkyl chains). HRMS (ESI) calc. for $C_{53}H_{90}N_2NaO_{11}S$, 1113.7365; found 1113.7343.

3-*O*-β-D-Glucopyranosyl-1,2-di-*O*-hexadecanoyl-*sn*-glycerol. A mixture of 3-*O*-(2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranosyl)-1,2-di-*O*-hexadecanoyl-*sn*-glycerol (0.488 g, 0.45 mmol) and 10% Pd/C (0.080 g) in 4:1 EtOH-CH_2Cl_2 (10 mL) was stirred for 16 hours under hydrogen pressure (5 atm.). After filtration through celite and concentration, the residue was purified by silica gel column chromatography (7:1 CH_2Cl_2-EtOH). Pure product was recovered as a solid in 88% yield. 0.290 g, R_f 0.45 (8:1 CH_2Cl_2-EtOH), mp 115°C, $[\alpha]_D^{20}$ -8.5 (*c* 1.0, $CHCl_3$) [lit^{25} $[\alpha]_D^{20}$ -11.7 (*c* 1.0, $CHCl_3$)]; ^{1}H NMR (2:1 $CDCl_3$-CD_3OD) : δ 5.26 (dddd, 1H, H-2$_{gly}$), 4.39 (dd, 1H, $J_{1agly,2gly}$ 3.0, $J_{1agly,1bgly}$ 12.0 Hz, H-1a$_{gly}$), 4.27 (d, 1H, $J_{1,2}$ 7.6 Hz, H-1), 4.20 (dd, 1H, $J_{1agly,2gly}$ 6.7 Hz, H-1b$_{gly}$), 3.92 (dd, 1H, $J_{2gly,3agly}$ 5.3, $J_{3agly,3bgly}$ 11.0 Hz, H-3a$_{gly}$), 3.85 (dd, 1H, $J_{5,6a}$ 2.4, $J_{6a,6b}$ 12.1 Hz, H-6a), 3.74 (dd, 1H, $J_{5,66}$ 6.1 Hz, H-6b), 3.72 (dd, 1H, $J_{2gly,3agly}$ 7.0 Hz, H-3b$_{gly}$), 3.44-3.37 (m, 2H, H-3, H-4), 3.32-3.21 (m, 2H, H-1, H-5), 2.36-2.27 (m, 4H, 2CH_2COO), 1.67-1.51 (m, 4H, 2CH_2CH$_2$COO), 1.38-1.15 (m, 48H, 24CH_2 alkyl chains), 0.88 (t, 6H, J 6.7 Hz, 2CH_3 alkyl chains); ^{13}C NMR (2:1 $CDCl_3$-CD_3OD): δ 173.98, 173.65 (C$_{15}$H$_{31}$*C*OO), 103.38 (C-1), 76.23, 76.10 (C-3, C-5), 73.34 (C-2), 70.21 (C-2$_{gly}$), 69.92 (C-4), 67.75 (C-3$_{gly}$), 62.72 (C-1$_{gly}$), 61.59 (C-6), 34.12, 33.98, 31.78, 29.55-28.94, 24.75, 22.52 (*C*H$_2$ alkyl chains), 13.80 (*C*H$_3$ alkyl chains). Anal. Calcd for $C_{41}H_{78}O_{10}$ (731.065): C, 67.36; H, 10.75. Found; C, 67.39; H, 10.75.

Synthesis of DP-α-Man

3-*O*-(2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyl)-1,2-*O*-isopropylidene-*sn*-glycerol. Obtained from 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl trichloracetimidate[26] (0.700 g, 1.42 mmol) and 1,2-*O*-isopropylidene-*sn*-glycerol (0.245 g, 1.86 mmol), following the same protocol as for the β-D-Glc derivative, in 64% yield after column chromatography (4:3 EtOAc-PE). Oily material; R_f 0.58 (4:3 EtOAc-PE); $[\alpha]_D^{20}$ +40.2 (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 5.33 (dd, 1H, $J_{2,3}$ 3.3, $J_{3,4}$ 10.3 Hz, H-3), 5.27 (dd, 1H, $J_{4,5}$ 9.5 Hz, H-4), 5.26 (dd, 1H, $J_{1,2}$ 1.2 Hz, H-2), 4.87 (d, 1H, H-1), 4.31-4.23 (m, 2H, H-6a, H-2$_{gly}$), 4.12 (dd, 1H, $J_{5,6b}$ 2.4, $J_{6a,6b}$ 12.3 Hz, H-6b), 4.08 (dd, 1H, $J_{1agly,2gly}$ 6.5, $J_{1agly,1bgly}$ 8.2 Hz, H-1a$_{gly}$), 4.03 (ddd, 1H, $J_{5,6a}$ 5.4 Hz, H-5), 3.94 (dd, 1H, $J_{3'a,3'b'}$ 10.5 Hz, H-3'a), 3.76 (dd, 1H, $J_{1bgly,2gly}$ 6.4 Hz, H-1b$_{gly}$), 3.71 (dd, 1H, $J_{2gly,3agly}$ 5.5, $J_{3agly,3bgly}$ 10.5 Hz, H-3a$_{gly}$), 3.56 (dd, 1H, $J_{2gly,3bgly}$ 5.4 Hz, H-3b$_{gly}$), 2.15, 2.10, 2.04, 1.98 (4s, 12H, 4CH_3COO), 1.42, 1.36 (2s, 6H, (CH_3)$_2$C); ^{13}C NMR ($CDCl_3$): δ 170.32, 169 .72, 169.68, 169.51 (CH_3COO), 109.39 (*C*(CH$_3$)$_2$), 97.46 (C-1), 74.07 (C-2$_{gly}$), 69.18 (C-2), 68.82 (C-3), 68.48 (C-5), 68.12 (C-3$_{gly}$), 65.89 (C-4), 66.14 (C-1$_{gly}$), 62.23 (C-6), 26.50, 25.29 ((*C*H$_3$)$_2$C), 20.64, 20.51, 20.47, 20.44 (*C*H$_3$COO). HRMS (ESI) calc. for $C_{20}H_{30}NaO_{12}$, 485.1629; found 485.1623.

3-*O*-(2,3,4,6-Tetra-*O*-benzyl-α-D-mannopyranosyl)-1,2-*O*-isopropylidene-*sn*-glycerol. Obtained from 3-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-1,2-*O*-isopropylidene-*sn*-glycerol (0.478 g, 1.055 mmol) following the same protocol as for the β-D-Glc derivative. The crude target compound was purified by silica gel column chromatography (2:5 EtOAc-PE): 72% yield; oily material; R_f 0.61; $[\alpha]_D^{20}$ +29.5 (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.42-7.17 (m, 20H, 4C$_6H_5$), 4.94 (d, 1H, $J_{1,2}$ 1.6 Hz, H-1), 4.90 and 4.54 (AB system, 2H, J 10.7 Hz, CH_2C$_6$H$_5$), 4.80 and 4.73 (AB system, 2H, J 12.5 Hz, CH_2C$_6$H$_5$), 4.68 and 4.57 (AB system, 2H, J 11.9 Hz, CH_2C$_6$H$_5$), 4.65 (s, 2H, CH_2C$_6$H$_5$), 4.25 (dddd, 1H, $J_{1agly,2gly}$ 6.5, $J_{1bgly,2gly}$ 6.6, $J_{2gly,3agly}$ 4.8, $J_{2gly,3bgly}$ 6.4 Hz, H-2$_{gly}$), 4.03 (dd, 1H, $J_{1agly,1bgly}$ 8.3 Hz, H-1a$_{gly}$), 4.01 (dd, 1H, $J_{3,4}$ 9.1, $J_{4,5}$ 9.4 Hz, H-4), 3.92 (dd, 1H, $J_{2,3}$ 3.0, H-3), 3.88 (dd, 1H, H-2), 3.81-3.74 (m, 3H, H-5, H-6a, H-6b), 3.70 (dd, 1H, $J_{3agly,3bgly}$ 10.5 Hz, H-3a$_{gly}$), 3.63 (dd, 1H, H-1b$_{gly}$), 3.49 (dd, 1H, H-3b$_{gly}$), 1.41, 1.38 (2s, 6H, (CH_3)$_2$C); ^{13}C NMR ($CDCl_3$): δ 138.36, 138.34, 138.23, 128.19-127.42 (*C*$_6$H$_5$), 109.34 (*C*(CH$_3$)$_2$), 98.19 (C-1), 79.85 (C-3), 74.79, 73.05, 72.39, 71.86 (*C*H$_2$C$_6$H$_5$), 74.63 (C-4), 74.38 (C-2, C-2$_{gly}$), 71.82 (C-5), 68.89 (C-6), 68.22 (C-3$_{gly}$), 66.27 (C-1$_{gly}$), 26.64, 25.36 ((*C*H$_3$)$_2$C). Anal. Calcd for $C_{40}H_{46}O_{08}$ (654.79): C, 73.37; H, 7.08. Found; C, 73.22; H, 7.06.

3-*O*-(2,3,4,6-Tetra-*O*-benzyl-α-D-mannopyranosyl)-*sn*-glycerol. Obtained from 3-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-mannopyranosyl)-1,2-*O*-isopropylidene-*sn*-glycerol (0.470 g, 0.72 mmol) following the same protocol as for the β-D-Glc derivative. The crude product was purified by silica gel column chromatography (1:2 ethyl EtOAc-PE). The target compound was obtained as an oily material in 90% yield. R_f 0.38 (1:1 EtOAc-PE), $[\alpha]_D^{20}$ +32.4 (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.30-7.15 (m, 20H, 4C$_6H_5$), 4.87-4.45 (m, 9H, 4CH_2C$_6$H$_5$, H-1), 4.15 (m, 1H, H-2$_{gly}$), 3.95-3.40 (m, 10H, H-2, H-3, H-4, H-5, H-6a, H-6b, H-1a$_{gly}$, H-1b$_{gly}$, H-3a$_{gly}$, H-3b$_{gly}$); ^{13}C NMR ($CDCl_3$): δ 138.52, 138.36, 138.13, 128.50-127.81 (*C*$_6$H$_5$), 98.88 (C-1), 80.05 (C-3), 75.15, 73.56, 72.87 72.39 (*C*H$_2$C$_6$H$_5$), 75.10, 75.00 (C-2, C-4), 72.18 (C-5), 70.73 (C-2$_{gly}$), 69.56, 69.47 (C-6, C-3$_{gly}$), 63.59 (C-1$_{gly}$). Anal. Calcd for $C_{37}H_{42}O_8$ (614.72): C, 72.29; H, 6.89. Found; C, 72.43; H, 6.88.

1,2-Di-*O*-hexadecanoyl-3-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-mannopyranosyl)-*sn*-glycerol. Obtained from 3-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-mannopyranosyl)-*sn*-glycerol (0.370 g, 0.60 mmol)

following the same protocol as for the β-D-Glc derivative. Purification by silica gel column chromatography (1:4 EtOAc-PE) afforded the target compound in 76% yield. Oily material; R_f 0.63; $[\alpha]_D^{20}$ +28.6 (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.42-7.15 (m, 20H, 4C$_6H_5$), 5.18 (dddd, 1H, $J_{1agly,2gly}$ 3.7, $J_{1bgly,2gly}$ 6.2, $J_{2gly,3agly}$ 4.9, $J_{2gly,3bgly}$ 5.6 Hz, H-2$_{gly}$), 4.89 (AB system, 1H, *J* 10.8 Hz, CH_2C$_6$H$_5$), 4.87 (bs, 1H, H-1), 4.77 and 4.71 (AB system, 2H, *J* 12.4 Hz, CH_2C$_6$H$_5$), 4.67-4.50 (m, 5H, 2.5CH_2C$_6$H$_5$), 4.32 (dd, 1H, $J_{1agly,1bgly}$ 11.8 Hz, H-1a$_{gly}$), 4.08 (dd, 1H, H-1b$_{gly}$), 4.01 (dd, 1H, $J_{3,4}$ 9.3, $J_{4,5}$ 9.7 Hz, H-4), 3.88 (dd, 1H, $J_{2,3}$ 3.0 Hz, H-3), 3.81 (dd, 1H, $J_{3agly,3bgly}$ 10.7 Hz, H-3a$_{gly}$), 3.79-3.70 (m, 4H, H-2, H-5, H-6a, H-6b), 3.54 (dd, 1H, H-3b$_{gly}$), 2.30 (t, 4H, *J* 6.8 Hz, 2CH_2COO), 1.66-1.53 (m, 4H, 2 CH_2CH$_2$COO), 1.38-1.20 (m, 48H, 24CH_2 alkyl chains), 0.89 (t, 6H, 2CH_3 alkyl chains); ^{13}C NMR ($CDCl_3$): δ 173.39, 173.07 (C$_{15}$H$_{31}$COO), 138.54, 138.48, 138.40, 138.36, 128.41-127.56 (C$_6$H$_5$), 98.47 (C-1), 80.02 (C-3), 75.14, 73.48, 72.83, 72.47 (CH$_2$C$_6$H$_5$), 74.97, 74.84 (C-2, C-4), 72.37 (C-5), 69.83 (C-2$_{gly}$), 69.23 (C-6), 65.71 (C-3$_{gly}$), 62.46 (C-1$_{gly}$), 34.35, 34.17, 32.03, 29.82-29.21, 25.07, 24.98, 22.80 (CH$_2$ alkyl chains), 14.24 (CH$_3$ alkyl chains). Anal. Calcd for $C_{69}H_{102}O_{10}$ (1091.54): C, 75.92; H, 9.42. Found; C, 76.19; H, 9.52.

1,2-Di-*O*-hexadecanoyl-3-*O*-α-D-mannopyranosyl-*sn*-glycerol. A mixture of 1,2-Di-*O*-hexadecanoyl-3-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-mannopyranosyl)-*sn*-glycerol (0.470 g, 0.430 mmol) and 10% Pd/C (0.080 g) in 1:1 CH_2Cl_2-EtOH (14 mL) was hydrogenated overnight under H_2 pressure (8 atm). After filtration through celite and concentration under reduced pressure, the residue purified by silica-gel column chromatography (8:1 CH_2Cl_2-EtOH). Pure product target compound was isolated in 80% yield. Mp 107°C, R_f 0.58; $[\alpha]_D^{20}$ +33.1 (*c* 1.0, 3:1 $CHCl_3$-MeOH); ^{1}H NMR (2:1 $CDCl_3$-CD_3OD): δ 5.20 (dddd, 1H, $J_{1agly,2gly}$ 3.3, $J_{1bgly,2gly}$ 6.3, $J_{2gly,3agly}$ 4.8, $J_{2gly,3bgly}$ 5.8 Hz, H-2$_{gly}$), 4.78 (d, 1H, $J_{1,2}$ 1.3 Hz, H-1), 4.36 (dd, 1H, $J_{1agly,1bgly}$ 12.0 Hz, H-1a$_{gly}$), 4.12 (dd, 1H, H-1b$_{gly}$), 3.83 (m, 1H, H-2), 3.81-3 .77 (m, 3H, H-3a$_{gly}$, H-6a, H-6b), 3.72 (dd, 1H, $J_{3,4}$ 9.4, $J_{4,5}$ 9.5 Hz, H-4), 3.70 (dd, 1H, $J_{2,3}$ 2.8 Hz, H-3), 3.58 (dd, 1H, $J_{3agly,3bgly}$ 10.6 Hz, H-3b$_{gly}$), 3.49 (ddd, 1H, $J_{5,6a}$ 3.0, $J_{5,6b}$ 2.8 Hz, H-5), 2.31 (dt, 4H, 2CH_2COO), 1.65-1.54 (m, 4H, 2CH_2CH$_2$COO), 1.37-1.20 (m, 48H, 24CH_2 alkyl chains), 0.87 (t, 6H, *J* 6.7 Hz, 2CH_3 alkyl chains); ^{13}C NMR (2:1 $CDCl_3$-CD_3OD): δ 173.80, 173.42 (C$_{15}$H$_{31}$COO), 100.29 (C-1), 72.72 (C-5), 71.09 (C-3), 70.34 (C-2), 69.81 (C-2$_{gly}$), 66.67 (C-4), 65.50 (C-3$_{gly}$), 62.41 (C-1$_{gly}$), 61.21 (C-6), 34.10, 33.94, 32.06, 26.53, 29.49, 29.34, 29.20, 28.96, 28.93, 28.88, 24.77, 24.72, 22.50 (CH$_2$ alkyl chains), 13.77 (CH$_3$ alkyl chains). Anal. Calcd for $C_{41}H_{78}O_{10}$,0.5H_2O (740.06): C, 66.54; H, 10.76. Found; C, 66.58; H, 10.72.

Synthesis of DP-β-(Dns)Glc

2,3,4-Tri-*O*-acetyl-6-azido-6-deoxy-D-glucopyranosyl trichloroacetimidate. Hydrazine acetate (1.105 g, 12.0 mmol), was added at 55°C to a solution of 1,2,3,4-tetra-*O*-acetyl-6-azido-6-deoxy-D-glucopyranose[27] (3.73 g, 10.0 mmol) in dry DMF (20 mL). After 10 min, the solution was allowed to cool to RT and stirring was maintained for 4 h. The mixture was poured into EtOAc (100 mL) and the organic phase was washed with brine (10 mL), dried (Na_2SO_4) and concentrated. The residue was coevaporated from toluene (3x10 mL). Trichloroacetonitrile (4 mL) and DBU (0.8 mL) were successively added at 0°C to solution of the crude product in CH_2Cl_2 (10 mL): the mixture was stirred for 2 h at 0°C and 2 h at rt. After concentration, the residue was purified by silica gel column chromatography (1:2 EtOAc-PE). Pure product[28] was isolated in 63% yield. 3.00 g, amorphous solid, R_f 0.53; $[\alpha]_D^{20}$ +114.8 (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 8.73 (s, 1H, N*H*), 6.58 (d, 1H, $J_{1,2}$ 3.7 Hz, H-1), 5.55 (dd, 1H, $J_{2,3}$ 10.2, $J_{3,4}$ 9.4 Hz, H-3), 5.15 (dd, 1H, $J_{4,5}$ 10.2 Hz, H-4), 5.13 (dd, 1H, H-2), 4.18 (ddd, 1H, $J_{5,6a}$ 2.8, $J_{5,6b}$ 5.4 Hz, H-5), 3.41 (dd, 1H, $J_{6a,6b}$ 13.5 Hz, H-6a), 3.33 (dd, 1H, H-6b), 2.06, 2.03, 2.01 (3s, 9H, 3CH_3COO); ^{13}C NMR ($CDCl_3$): δ 170.14, 169.93, 169.64 (CH$_3$*C*OO), 160.79 (*C*=NH), 92.79 (C-1), 90.79 (*C*Cl$_3$), 71.26, 69.82, 69.80, 69.03 (C-2, C-3, C-4, C-5), 50.72 (C-6), 20.81, 20.75, 20.57 (*C*H$_3$COO). HRMS (ESI) calc. for $C_{14}H_{17}Cl_3N_4NaO_8$, 497.0004; found 497.9992.

3-*O*-(6-Azido-2,3,4-tri-*O*-benzyl-6-deoxy-β-D-glucopyranosyl)-1,2-*O*-isopropylidene-*sn*-glycerol. Obtained from 2,3,4-tri-*O*-acetyl-6-azido-6-deoxy-D-glucopyranosyl trichloroacetimidate (0.490 g, 1.10 mmol) following the same protocol as for the α-D-Man derivative. The crude residue was purified by column chromatography (1:5 EtOAc-PE): 81% yield; solid: mp 111-112°C; R_f 0.56; $[\alpha]^{20}_D$ +18.0 (c 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.40-7.24 (m, 15H, 3C$_6H_5$), 4.99-4.55 (m, 6H, 3CH_2C$_6$H$_5$), 4.49 (d, 1H, $J_{1,2}$ 7.7 Hz, H-1), 4.35 (dddd, 1H, $J_{1agly,2gly}$ 6.4, $J_{1bgly,2gly}$ 6.2, $J_{2gly,3agly}$ 5.0, $J_{2gly,3bgly}$ 5.4 Hz, H-2$_{gly}$), 4.08 (dd, 1H, $J_{1agly,1bgly}$ 8.2 Hz, H-1a$_{gly}$), 4.01 (dd, 1H, $J_{3agly,3bgly}$ 10.3 Hz, H-3a$_{gly}$), 3.85 (dd, 1H, H-1b$_{gly}$), 3.70-3.30 (m, 7H, H-2, H-3, H-4, H-5, H-6a, H-6b, H-3b$_{gly}$), 1.43, 1.38 (2s, 6H, (CH_3)$_2$C); ^{13}C NMR ($CDCl_3$): δ 138.50, 138.43, 137.83, 128.63-127.81 (C$_6$H$_5$), 109.58 (*C*(CH$_3$)$_2$), 103.76 (C-1), 84.49 (C-3), 82.24 (C-2), 78.32 (C-4), 75.79, 75.20, 74.78 (CH$_2$C$_6$H$_5$), 74.82 (C-5), 74.45 (C-2$_{gly}$), 70.34 (C-3$_{gly}$), 66.75 (C-1$_{gly}$), 51.47 (C-6), 26.90, 25.47 (C(*C*H$_3$)$_2$). Anal. Calcd for $C_{33}H_{39}N_3O_7$ (598.68): C, 67.22; H, 6.67; N, 7.13. Found: C, 67.00; H, 6.65; N, 6.92.

3-*O*-(6-Azido-2,3,4-tri-*O*-benzyl-6-deoxy-β-D-glucopyranosyl)-*sn*-glycerol. Obtained from 3-*O*-(6-azido-2,3,4-tri-*O*-benzyl-6-deoxy-β-D-glucopyranosyl)-1,2-*O*-isopropylidene-*sn*-glycerol (0.450 g, 0.75 mmol) following the same protocol as for the α-D-Man derivative. The crude residue was purified by silica gel column chromatography (2:1 EtOAc-PE). Pure target compound was isolated in 81% yield as a solid: 0.340 g. R_f 0.53; mp 93°C; $[\alpha]^{20}_D$ +14.0 (c 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.40-7.24 (m, 15H, 3C$_6H_5$), 5.00-4.59 (m, 6H, 3CH_2C$_6$H$_5$), 4.47 (d, 1H, $J_{1,2}$ 7.8 Hz, H-1), 3.90-3.28 (m, 11H, H-2, H-3, H-4, H-5, H-6a, H-6b, H-1a$_{gly}$, H-1b$_{gly}$, H-2$_{gly}$, H-3a$_{gly}$, H-3b$_{gly}$); ^{13}C NMR ($CDCl_3$): δ 138.42, 138.37, 137.79, 128.69-127.87 (C$_6$H$_5$), 103.82 (C-1), 84.51 (C-3), 82.21 (C-2), 78.28 (C-4), 75.83, 75.10, 75.20 (CH$_2$C$_6$H$_5$), 74.59 (C-5), 72.07 (C-3$_{gly}$), 70.91 (C-2$_{gly}$), 63.65 (C-1$_{gly}$), 51.42 (C-6). Anal. Calcd for $C_{30}H_{35}N_3O_7$ (549.61): C, 65.56; H, 6.42; N, 7.65. Found: C, 65.48; H, 6.64; N, 7.49.

3-*O*-(6-Azido-2,3,4-tri-*O*-benzyl-6-deoxy-β-D-glucopyranosyl)-1,2-di-*O*-hexadecanoyl-*sn*-glycerol. Obtained from 3-*O*-(6-azido-2,3,4-tri-*O*-benzyl-6-deoxy-β-D-glucopyranosyl)-*sn*-glycerol (0.302 g, 0.55 mmol) following the same protocol as for the β-D-Glc derivative. Purification by silica gel column chromatography (1:4 EtOAc-PE): 84% yield. Amorphous solid; R_f 0.71; $[\alpha]^{20}_D$ +14.0 (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.39-7.20 (m, 15H, $3C_6H_5$), 5.28 (dddd, 1H, $J_{1agly,2gly}$ 3.8, $J_{1bgly,2gly}$ 6.6, $J_{2gly,3agly}$ 4.4, $J_{2gly,3bgly}$ 4.5 Hz, H-2_{gly}), 4.96-4.53 (m, 6H, $3CH_2C_6H_5$), 4.43 (d, 1H, $J_{1,2}$ 7.7 Hz, H-1), 4.37 (dd, 1H, $J_{1agly,1bgly}$ 11.8 Hz, H-$1a_{gly}$), 4.23 (dd, 1H, H-$1b_{gly}$), 4.05 (dd, 1H, $J_{3agly,3bgly}$ 10.8 Hz, H-$3a_{gly}$), 3.72 (dd, 1H, H-$3b_{gly}$), 3.64 (dd, 1H, $J_{2,3}$ 8.6 Hz, $J_{3,4}$ 8.6 Hz, H-3), 3.52-3.40 (m, 3H, H-2, H-4, H-5), 3.40-3.37 (m, 1H, H-6a), 3.29 (dd, 1H, $J_{5,6b}$ 5.8, $J_{6a,6b}$ 13.0 Hz, H-6b), 2.28 (q, 4H, *J* 6.8 Hz, $2CH_2COO$), 1.70-1.55 (m, 4H, $2CH_2CH_2COO$), 1.45-1.20 (m, 48H, $24CH_2$ alkyl chains), 0.89 (t, 6H, *J* 6.7 Hz, $2CH_3$ alkyl chains); ^{13}C NMR ($CDCl_3$): δ 173.50, 173.16 (CH_2COO), 138.47, 138.34, 137.79, 128.65-127.82 (C_6H_5), 103.71 (C-1), 84.39 (C-3), 82.05 (C-2), 78.26 (C-4), 75.83, 75.25, 74.90 ($CH_2C_6H_5$), 74.95 (C-5), 70.03 (C-2_{gly}), 68.07 (C-3_{gly}), 62.66 (C-1_{gly}), 51.41 (C-6), 34.40, 34.22, 32.08, 29.86-29.23, 25.05, 22.84 (CH_2 alkyl chains), 14.27 (CH_3 alkyl chains). Anal. Calcd for $C_{62}H_{95}N_3O_9$ (1026.43): C, 72.55; H, 9.33; N, 4.09. Found: C, 72.48; H, 9.57; N, 3.86.

3-*O*-(6-Dansylamino-6-deoxy-β-D-glucopyranosyl)-1,2-di-*O*-hexadecanoyl-*sn*-glycerol. 3-*O*-(6-Azido-2,3,4-tri-*O*-benzyl-6-deoxy-β-D-glucopyranosyl)-1,2-di-*O*-hexadecanoyl-*sn*-glycerol (0.308 g, 0.30 mmol) in 1:1 CH_2Cl_2-EtOH (8 mL) was hydrogenated under H_2 pressure (6 atm) in the presence of 10% Pd/C (100 mg). After 16 h and filtration through celite, the solution was concentrated under reduced pressure, affording quantitatively the de-*O*-benzylated 6-amino-6-deoxy derivative as an oily material. $[\alpha]^{20}_D$ 4.6 (*c* 1.0, $CHCl_3$); ^{13}C NMR ($CDCl_3$): δ 173.97, 173.73 ($C_{15}H_{31}COO$), 103.14 (C-1), 73.17, 71.59, 71.29, 70.23 (C-2, C-3, C-4, C-5, C-2_{gly}), 67.87 (C-3_{gly}), 63.00 (C-1_{gly}), 40.87 (C-6), 34.17, 34.00, 31.80, 29.59-29.05, 24.81, 22.54 (CH_2 alkyl chains), 13.88 (CH_3 alkyl chains). A mixture of the 6-amino derivative (0.098 g, 0.134 mmol) and dansyl chloride (0.049 g, 0.182 mmol) in $CHCl_3$ (3 mL) was vigorously stirred in the presence of a $NaHCO_3$ solution [(0.027 g, 0.321 mmol) in 0.5 mL H_2O]. After 16 h, the aqueous phase was diluted with H_2O (4 mL) and extracted with $CHCl_3$ (3x5 mL). The combined organic phases were dried, concentrated and the residue was purified on silica-gel column chromatography (4:1 EtOAc-PE): 0.074 g, 58% yield. R_f 0.71; $[\alpha]^{20}_D$ –25.3 (*c* 1.0, $CHCl_3$); ^{1}H NMR (5:2 $CDCl_3$-CD_3OD): δ 8.57 (d, 1H, *J* 8.4 Hz, H naphtyl), 8.35 (d, 1H, *J* 8.6 Hz, H naphtyl), 8.24 (d, 1H, *J* 7.1 Hz, naphtyl H), 7.59-7.54 (m, 2H, naphtyl H), 7.25-7.23 (d, 1H, *J* 7.5 Hz, naphtyl H), 5.21 (m, 1H, H-2_{gly}), 4.36 (dd, 1H, $J_{1agly,2gly}$ 2.6, $J_{1agly,1bgly}$ 12.1 Hz, H-$1a_{gly}$), 4.20-4.15 (m, 1H, H-$1b_{gly}$), 4.05 (d, 1H, $J_{1,2}$ 7.7 Hz, H-1), 3.74 (dd, 1H, $J_{2',3'a}$ 5.0, $J_{3agly,3bgly}$ 10.8 Hz, H-$3a_{gly}$), 3.60 (dd, 1H, $J_{2gly,3bgly}$ 2.6 Hz, H-$3b_{gly}$), 3.42-3.01 (m, 6H, H-2, H-3, H-4, H-5, H-6a, H-6b), 2.92 (s, 6H, $(CH_3)_2N$), 2.34 (q, 4H, *J* 7.4 Hz, $2CH_2COO$), 1.68-1.55 (m, 4H, $2CH_2CH_2COO$), 1.40-1.20 (m, 48H, $24CH_2$ alkyl chains), 0.88 (t, 6H, *J* 6.8 Hz, $2CH_3$ alkyl chains); ^{13}C NMR (5:2 $CDCl_3$-CD_3OD): δ 173.78, 173.49 (CH_2COO), 151.85 (C-SO_2), 135.16, 129.91, 129.62 (*C*-quat), 130.39, 129.31, 128.35, 123.35, 119.12, 115.27 (*C*-naphtyl), 103.45 (C-1), 75.76, 74.35, 73.49, 70.32, 70.15 (C-2, C-3, C-4, C-5, C-2_{gly}), 68.26 (C-3_{gly}), 63.02 (C-1_{gly}), 45.48 ($N(CH_3)_2$), 43.75 (C-6), 34.35, 34.22, 31.98, 29.77-29.18, 24.95, 22.74 (CH_2 alkyl chains), 14.17 (CH_3 alkyl chains). HRMS (ESI) calc. for $C_{53}H_{90}N_2O_{11}S$, 963.6344 ; found 963.6345.

Synthesis of peptidyl RNA

For the synthesis of peptidyl RNA the foremost N^6-dibutylformamidine derivative of 5'-O-DMT-3'-N-Fmoc-L-alanylamino-3'-deoxyadenosine was immobilised through a 3,6,9-trioxaundecanoic diacid spacer on *200 Amino*™ solid support (GE Healthcare, 150 µmol DMT^+/g resin). The peptide synthesis was carried out on a peptide synthesiser equipped with UV monitoring at 303 nm. Fmoc was deprotected with 5 % piperidine in DMF, followed by coupling under *Fastmoc*® conditions, no capping was applied. An aliquot of the resin-bound peptide was deblocked with 5 % piperidine and solubilised with 33 % ethanolic methylamine. The crude purity and identity (exact mass) of $LA_3LA_2LA_3LA_2LA_3LA$-3'-amino-3'-deoxyadenosine was obtained from RP-HPLC coupled to ESI-MS ToF in the positive ion mode. HRMS: calc. for $C_{88}H_{151}N_{26}O_{23}$ 1940.1440, found 1940.1438, *cf.* Figs. S1–S4.† Fully protected and resin-bound 5'-O-DMT-3'-N-Fmoc-peptidylamino-3'-deoxyadenosine was transferred to a 1 µmole reaction column of a DNA/RNA synthesiser. A DMT^+-monitored RNA synthesis of the sequence 5'-GGGGCUCUUXGGAGCUCCACCA-3', where X = C or T(FAM), *cf* Fig. 1c, was carried out using commercial highly base-labile nucleoside 2'-O-TBDMS CE phosphoramidites, according to a previously described protocol for the solid support synthesis and isolation of pure amphiphilic peptidyl-RNA.[15] The target compound was identified through its exact mass by RP-HPLC coupled to ESI-MS ToF in the negative ion mode. HRMS: calc. for $C_{136}H_{422}N_{104}O_{179}P_{21}$ 9187.1606, found $[M - k\cdot H + l\cdot Na + m\cdot K + n\cdot PO_4]^z$ for z = –4 to –13, *cf.* Figs. S5–S7.†

Natural swelling of dry lipids to give GVs

A $CHCl_3$/MeOH 4:1 *v/v* solution of the phospholipid POPC, a mixture of phospholipids DOPC/DPPC 1:4 or of other amphiphilic compounds DOPC/DPPC/CHOL 1:3:1 ; POPC/oleic acid 1:2 ; POPC/glycolipid 95–80:5–20, mostly containing 0.2–0.01 mol % DOPE-Rh (or DPPE-Rh), was deposited on the glass wall of a round bottom flask through complete evaporation of the volatiles using a rotatory evaporator. The resulting thin lipidic film was hydrated with aqueous buffer (volume to 0.5 mM total amphiphile) of defined osmolality (100 mM sucrose) and pH 7.4 (10 mM Tris·HCl) during 1–4 days. To assure the hydration of all lipidic amphiphiles into disordered liquid phases, natural swelling was kept at 37 °C or, for certain DPPC or CHOL containing mixtures, at 65 °C. The less shaken or stirred the suspensions were during hydration the larger the multilamellar and multivesicular vesicles became, in our case giant. Before being transferred to wells of microscope plates and incubated with externally added agents (calcein, RNA, peptidyl RNA), the fully hydrated suspension was isotonically 5-fold diluted with 100 mM glucose (containing 10 mM Tris·HCl) to a final 0.1 mM total amphiphile concentration.

Incubation and analysis of GVs

For fluorescence imaging 30 μL 0.1 mM lipid suspension were treated with 1 μL 100 μM calcein (total molar lipid/calcein ≈30:1), 1 μL 10 μM RNA(FAM) (total molar lipid/RNA(FAM) ≈300: 1), or 1 μL 20 μM, 10 μM or 2 μM peptidyl-RNA(FAM), respectively; $\varepsilon_{260\ nm}$(RNA) = 205.3 (mM cm)$^{-1}$: total molar lipid/peptidyl-RNA(FAM) ≈150:1, 300:1, 1500:1. The well plates were imaged within 1–180 minutes; charged well plates were stocked for ageing studies at 4 °C and re-imaged after 4–18 hours, some after 3–7 days. In multiple controls, where sucrose and glucose had been replaced by pure water, no difference in size distribution, shape or other property or behavior of the vesicles was observed, except for their generally much higher thermal translational mobility under the microscope, which severly limited the quality of imaging.

Droplet transfer and encapsulation into GVs

In an Eppendorf tube (1.5 mL) were overlayed, in the following order (without mixing): (*i*) 500 μL of 10 mM Tris·HCl (pH 7.5)/100 mM glucose; (*ii*) 500 μL of 0.5 mM sodium oleate, 0.5 mM POPC in mineral oil; (*iii*) 500 μL of a water-in-oil emulsion prepared by emulsifying (i.e., pipetting up and down) 10 μL 5 μM calcein in 10 mM Tris·HCl (pH 7.5)/100 mM sucrose in 1 mM POPC in mineral oil. In come cases, the solutions contained peptidyl-RNA(FAM) (1 μM) instead of calcein. The tube was immediately centrifuged at 295 *g* for 10 minutes at room temperature. After centrifugation, the mineral oil was removed, and the aqueous phase was centrifuged at 1844 *g* for 10 minutes at room temperature. GVs, present as visible pellets on the bottom of the tube, were resuspended in 200 μL of 10 mM Tris·HCl (pH 7.5)/100 mM glucose. In most of the experiments 0.01 mol % DOPE-Rh were mixed with the lipids in both mineral oil solutions.

Confocal laser scanning fluorescence images

More than 2000 confocal fluorescence laser scanning microscopy images taken through three superimposable channels (green, red, and bright light) were recorded and analysed (Figs. S10–S12, Equations S1–S13, Tables S1–S4), in order to localise and quantify lipid-anchored peptidyl RNA (Fig. S9). The most representative or informative ~200 images were compiled into Image Files S1–S8.† A small group of the scientifically most revealing images are depicted in Figs. 3 and 4.

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Notes and references

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† Electronic Supplementary Information (ESI) available: Synthetic and imaging protocols, MS and NMR spectra (60 printable pages). Detailed descriptions of all imaging experiments, including control experiments with RNA(FAM) and peptidyl-NHdA, as well as the estimated water-lipid partitioning and ring profile distribution analysis for peptidyl-RNA-(FAM), are given in section 12. All membrane permeability experiments, including an increase in proton permeation caused by the addition of peptidyl RNA or peptidyl-NHdA to POPC LUVs, are given in section 13. The CD and IR analyses are detailed in sections 14, 15 and 16. Additional high-resolution confocal fluorescence image files IF S1, IF S3 and IF S4 are downloadable PDF documents meant for on-screen viewing only, they can be zoomed-in up to 5000 %. See DOI: 10.1039/c000000x/

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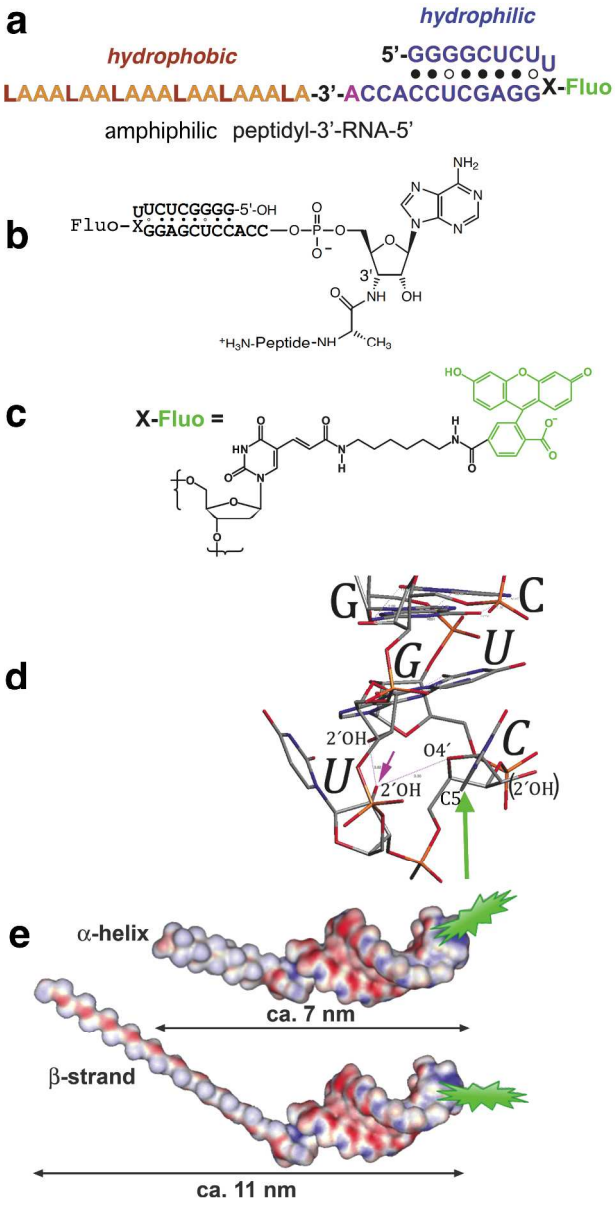

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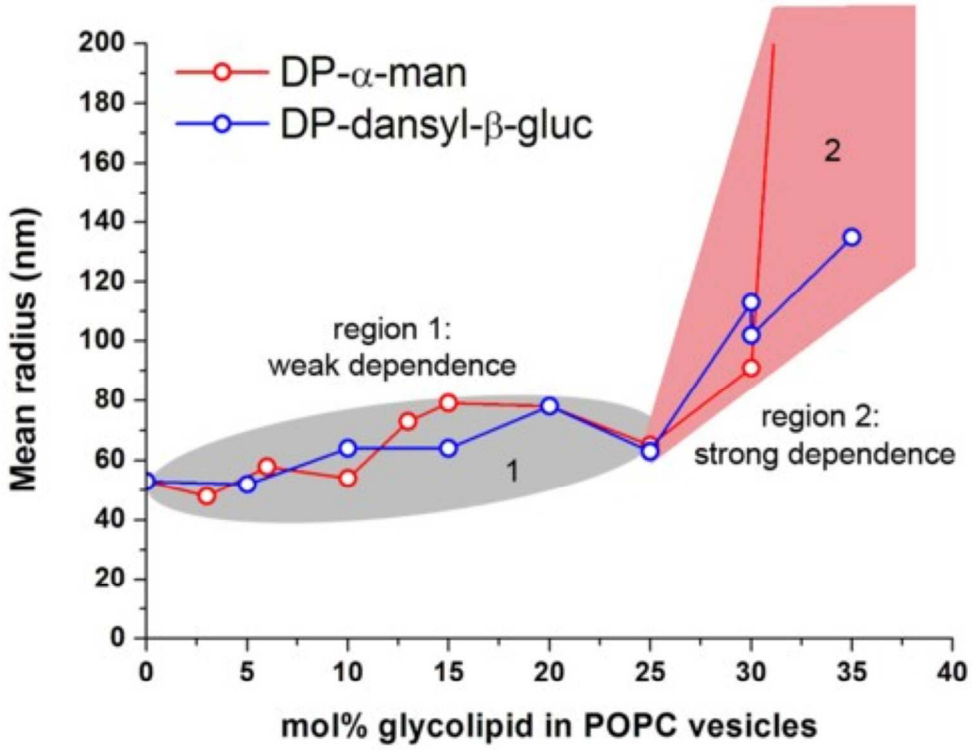

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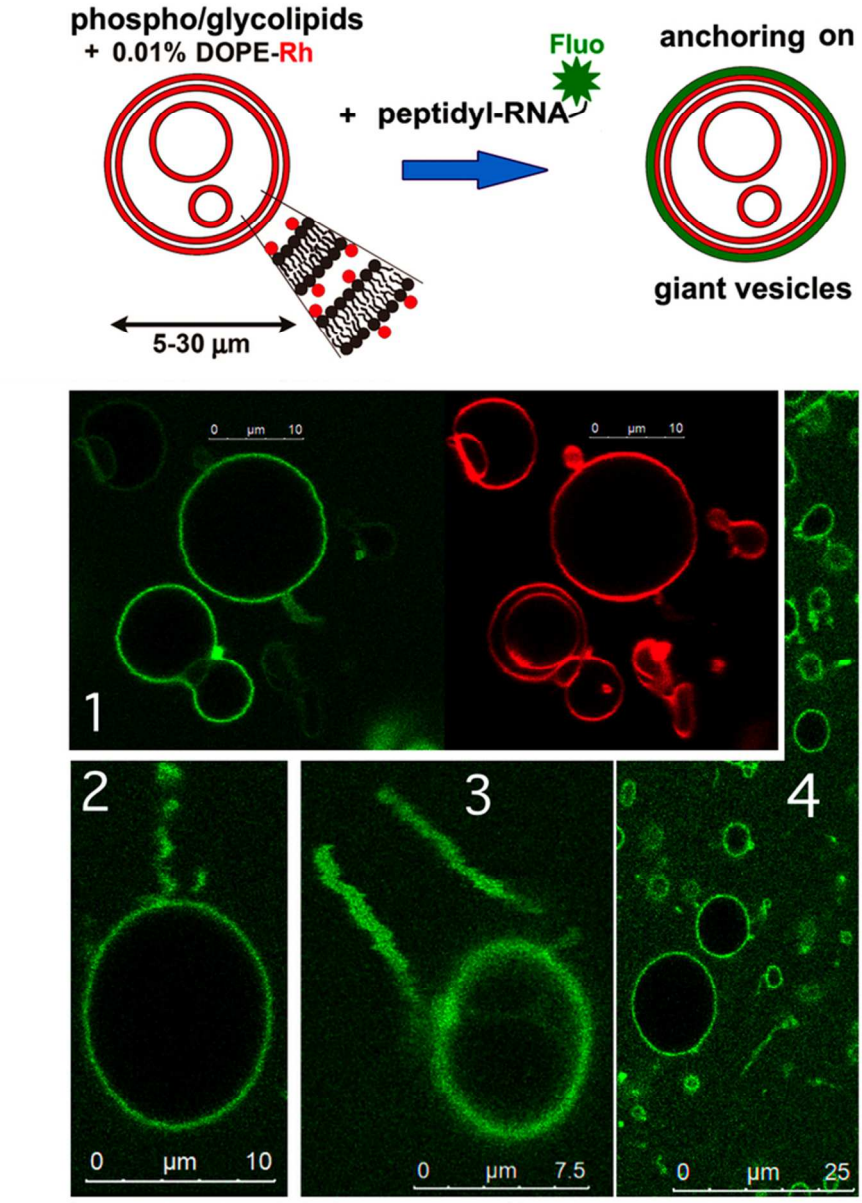

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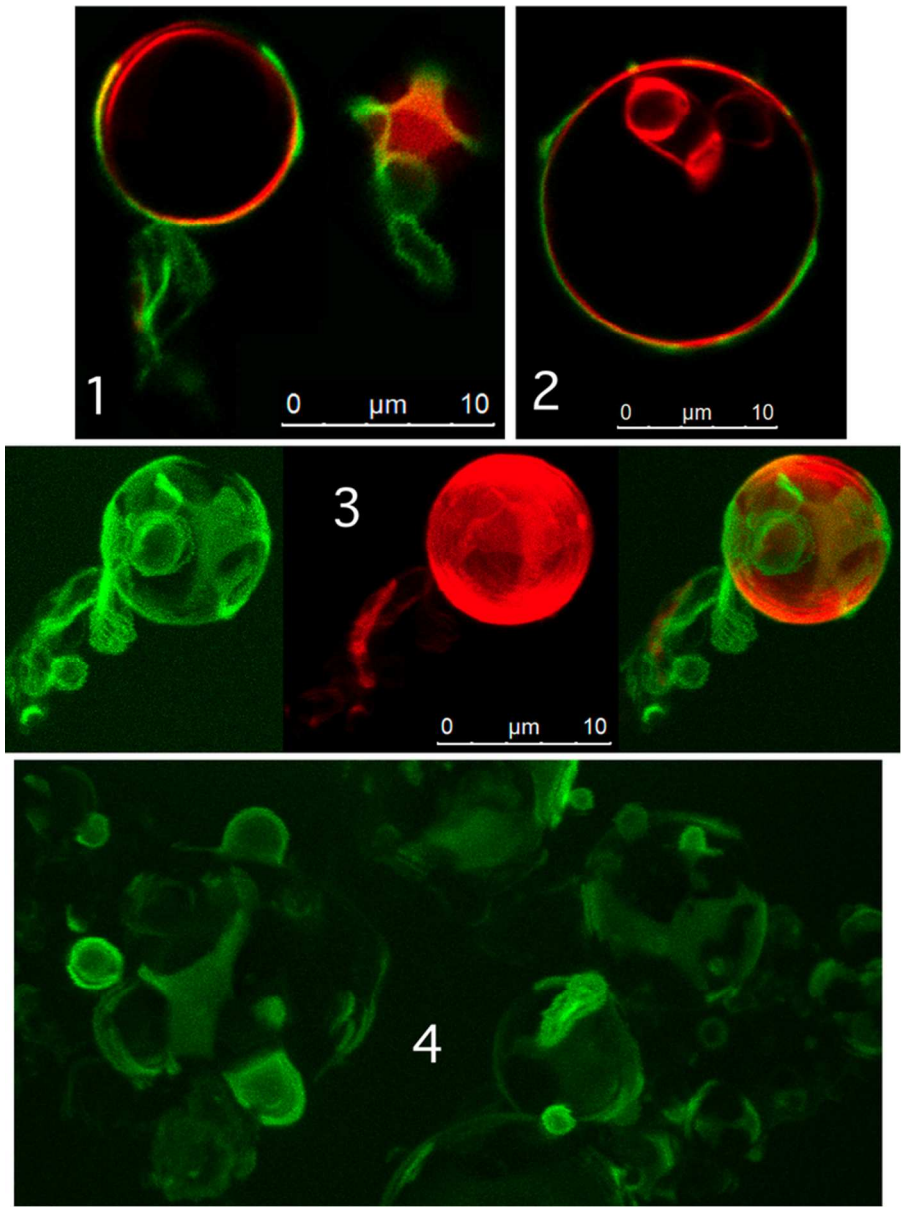

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