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Conformationally restricted monosaccharide-cored glycoside amphiphiles: the effect of detergent head group variation on membrane protein stability

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Abstract

Detergents are widely used to isolate membrane proteins from lipid bilayers, but many proteins solubilized in conventional detergents are structurally unstable. Thus, there is major interest in the development of novel amphiphiles to facilitate membrane protein research. In the present study, we have designed and synthesized novel amphiphiles with a rigid scyllo-inositol core, designated scyllo-inositol glycosides (SIGs). Varying the head group structure allowed the preparation of three sets of SIGs that were evaluated for their effects on membrane protein stability. When tested with a few model membrane proteins, representative SIGs conferred enhanced stability to the membrane proteins compared to a gold standard conventional detergent (DDM). Of the novel amphiphiles, a SIG designated STM-12 was most effective at preserving the stability of the multiple membrane proteins tested here. In addition, a comparative study of the three sets suggests that several factors including micelle size and alkyl chain length need to be considered in the development of novel detergents for membrane protein research. Thus, this study not only describes new detergent tools potentially useful for membrane protein structural study, but also introduces plausible correlations between the chemical properties of detergents and membrane protein stabilization efficacy.

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ASSOCIATED CONTENT

The supporting information is available free of charge via the internet at http://pubs.acs.org, including Figures S1 through S5, supplementary methods on detergent evaluation with membrane proteins, and synthetic protocols and characterizations of the new detergents.

Conflicts of Interest. The authors declare the following competing financial interest(s): P.S.C. and A.S. are co-inventors on a patent application that covers the SIG agents.

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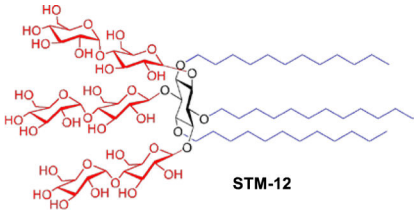

Keywords

facial amphiphile; membrane proteins; protein structure; protein stabilization; detergent micelles

Transcripted by 30% genome[1], membrane-embedded proteins are fundamental to a variety of cellular process including signal reception, transduction, material transportation, catalysis and energy interconversion.[2] Impairments of these bio-macromolecules are associated with various health disorders (developmental, immune, oncogenic, neuro-degenerative and cardiovascular diseases),[3] illustrating the fact that 60% of the currently available therapeutic agents target these membrane proteins.[4] Thus, the precise structural information of membrane proteins is of significance for fundamental understandings of their biological functions as well as for protein structure-based drug development.[5] However, atomic level structural analysis of membrane proteins remained an unconquered area until 1985, when Deissenhofer *et al.*, described the crystal structure of the bacterial photosynthetic reaction center-detergent complex.[6] Since this breakthrough, achieved using LDAO (lauryldimethylamine-*N*-oxide) as a detergent tool, protein-detergent complexes (PDCs) have been a mainstream approach for membrane protein structural study. Despite tremendous efforts, however, membrane proteins accounts for only 1~2 % of the total entries in PDB,[7] indicating the presence of multiple hurdles associated with membrane protein structural study. Membrane proteins are amphipathic in nature, which renders them unstable outside lipid bilayers. These biomacromolecules are also dynamic, an inherent attribute necessary for their cellular functions, making them recalcitrant to crystallization. Detergents have an ability to dismantle lipid bilayers and assemble around membrane proteins to form PDCs.[8] One major challenge in the structural study is to retain structural integrity of membrane proteins over the course of protein extraction, purification and crystallization. Finding a detergent stabilizing a target membrane protein over the course of protein manipulation, therefore, is critically important for *in vitro* functional and structural analyses, [9] including single particle cryoEM.[10]

The early success in membrane protein structural determination mainly relied on the use of a few non-ionic conventional detergents. Single head and tail group-bearing detergents such as

OG (*n*-octyl-β-D-glucoside), DM (*n*-decyl-β-D- maltoside), and DDM (*n*-dodecyl-β-D-maltoside)) have largely contributed to the structure determinations of membrane proteins.[11] These agents are still among the most widely used detergents for membrane protein manipulation. However, many membrane proteins, particularly mammalian proteins, tend to lose their structural integrity when treated with these agents.[12] The canonical architecture of conventional detergents, that is, a single head and tail groups, has a serious limitation in coping with a large number of membrane proteins with diverse structures/functions. Therefore, development of novel detergents with enhanced protein stabilization efficacy is of pivotal importance to advance membrane protein science.[13]

The past two decades have witnessed a modest growth in the development of membrane mimetic systems designed to overcome the limitations of conventional detergents.[14] Bicelles,[15] nanodiscs (NDs),[16] hemi-fluorinated surfactants (HFSs),[17] polymeric amphiphiles (amphipols (Apols) and styrene-maleic acid co-polymers (SMAs))[18,19] and peptide-based amphiphiles (lipopeptides (LPDs) and β-peptides (BPs))[20,21] have been invented as alternatives to conventional detergents. Parallel to this development, there has been a surge in the development of small amphiphilic compounds distinct from the canonical structure of conventional detergents. Representatives include tripod amphiphiles (TPAs),[22] xylene-linked maltoside amphiphiles (XMAs),[23] resorcinarene-based glucoside amphiphiles (RGAs),[24] norbornane-based amphiphiles (NBMs),[25] neopentyl glycol (NG)-based amphiphiles (GNGs, MNGs and NDTs),[26–28] penta-saccharide-bearing amphiphiles (PSEs)[27] and dendronic trimaltosides (DTMs).[30] These novel agents contain multiple head and tail groups introduced into detergent scaffolds *via* branch points in the hydrophilic/hydrophobic region. Some novel amphiphiles have single large lipophilic groups such as cholesterol (chobimalt),[31] diosgenin (GDN)[32] and cholate (facial amphiphiles (FAs)).[33] Of these small amphiphiles, NG class,[26,27] FAs[33] and GDN[32] have contributed in establishing high resolution 3D structures of ~40 membrane proteins including β_2 adrenergic receptor,[34] acetylcholine,[35] and dimeric F_o region of mitochondrial ATP synthase[36] in the last several years. This remarkable contribution has encouraged chemists to develop innovative amphiphiles to further facilitate membrane protein structural study. In the present study, we exploited a highly symmetrical monocyclic carbohydrate (i.e., scyllo-inositol) as a central core to design monosaccharide-cored tripod amphiphiles, designated scyllo-inositol-cored glycosides (SIGs). These new agents consist of three alkyl chains and three/six carbohydrate-based head groups connected *via* the central scyllo-inositol linker. These new amphiphiles showed a large variation in self-assembly behaviors depending on head group architecture. When tested with multiple model membrane proteins, a couple of the new agents displayed favorable properties for both protein solubilization and stabilization compare to DDM.

RESULTS AND DISCUSSION

Detergent structures and physical characterizations

Scyllo-inositol (SI) used for the core of new amphiphiles is a naturally occurring polyhydroxy cyclohexane. This monosaccharide molecule could adopt a conformation having all six hydroxy groups in either equatorial or axial positions. Due to thermodynamic

constraints, this carbocylic carbohydrate exclusively exists as a conformational isomer with all hydroxy groups in the equatorial position, thereby giving a unique 1,3,5/2,4,6-facial segregation of the substituents around the cyclohexane ring (Figure 1).[37] By introducing different alkyl chains and carbohydrates into 1,3,5 and 2,4,6-positions, respectively, we prepared SI-cored amphiphiles with the tail and head groups in an alternating arrangement with respect to the central ring.[38] Depending on the identity of the head group (glucose/maltose) and the presence/absence of a linker, these new agents can categorized into three sets. Three maltose groups were attached to the 2,4,6-hydroxy groups of SI directly (SI-cored trimaltosides (STMs)) or *via* ethylene glycol linker (STM-Es). Alternatively, using glycerol linker, six glucose units could be connected to the core ring (SHG-Gs). Depending on the hydrophilicity of the head group, different length ranges of alkyl chains were introduced into the other positions of the core ring (*i.e.*, 1,3,5-positions). Four different alkyl chains (C7, C8, C9, and C10) were introduced for the preparation of the STM-Es, while three different alkyl chains (C10, C11 and C12) were attached for STM preparations. As for the preparation of the SHG-Gs, three long alkyl chains (C12, C13 and C14) could be introduced to build the hydrophobic face thanks to the presence of the hexa-glucoside head group with a large hydrophilicity. Thus, we can attain a large variation in alkyl chain length from C7 to C14 by the systematic variation in the detergent hydrophilic group, which was indicated in detergent designation. This chain length variation is necessary to have a detergent with an optimal hydrophile-lipophile balance (HLB) that is known to be crucial for detergent efficacy for protein stabilization.[39]

The current amphiphiles can be categorized into tripod amphiphiles because of the presence of three alkyl chains in the lipophilic region. However, the current tripod amphiphiles differ from previous versions (TPAs and TMNs).[22,40] The previous tripod amphiphiles have branching points localized in both hydrophilic and hydrophobic portions rather than at the central part of the molecules. As the branching points were connected *via* a thin alkyl spacer, these previous versions likely contain large empty spaces in their micelle interiors, particularly in the hydrophilic-hydrophobic interface. Due to the presence of the monosaccharide core in this interfacial region, the SIGs would form micelles with fewer empty spaces in their interiors, resulting in an effective micellar packing favorably associated with membrane protein stability. In addition, the new agents would have high hydrophobic density mainly since the multiple alkyl chains were fabricated onto one face of the SI ring. This high hydrophobic density and the presence of the multiple alkyl chains likely to contribute to an increase in detergent-protein interactions. Due to the high preference of the SI ring for the conformer with all equatorial substituents, in addition, the central parts of the new agents are significantly rigid in their conformations compared to conventional detergents. Combined with the pre-organization of the head and tail groups into opposite faces of the core ring, this structural feature is likely to induce enhanced membrane protein stabilization and may have positive effects on crystallization. Thus, multiple detergent characteristics, mainly originating from the introduction of the conformationally restricted SI ring in the central region, are to play important roles in effective protein solubilization as well as stabilization.

These scyllo-inositol-cored glycosides were synthesized *via* a multistep-synthetic protocol starting from either orthoester-protected scyllo-inositol (**1a**) or its allyl group-conjugated derivative (**1b**) (Scheme S1). These protected scyllo-inositol derivatives (**1a** and **1b**) are highly symmetrical (D_{3d}) and were prepared from inositol with a σ1 symmetry according to a reported methodology.[41] A simplicity of peaks in ^{1}H-NMR spectra is in accordance with the highly symmetric chemical structures of these derivatives (Figure S1). After attaching three alkyl chains into the free hydroxy groups of compound **1a**, the orthoester protecting group was removed to furnish 1,3,5-trialkylated triol derivatives (**A**). Orthoester-deprotection of compound **1b** followed by tri-alkylation of the SI ring at 1,3,5-OH positions afforded tri-allylated intermediates (**B**) containing different lengths of alkyl chains. The three allyl groups of these intermediates were utilized to generate ethylene glycol (**C**)/ glycerol linker (**D**) *via* an ozone (O_3)/OsO_4-based oxidation reaction. The resulting tri-alkylated SI derivatives with multiple alcohol groups (**A**, **C** and **D**) were further elaborated by glycosylation and subsequent global deprotection to yield the STMs, STM-Es and SHGs-Gs, respectively. As we used benzoyl-protected glucosyl/maltosyl bromide as a glycosyl donor in the glycosylation reaction, a newly formed glycosidic bond is likely to have β −stereochemistry. The anomeric purities of these amphiphiles were supported by their ^{1}H NMR spectra. STM-11 having the direct connection of the maltoside head group to the SI ring showed an anomeric peak at 4.79 ppm as a doublet with a coupling constant (*J*) of 8.0 Hz (Figure. 2a & S2a). These chemical shift and large coupling constant indicate the formation of β-glycosidic bond. We also observed another anomeric peak at 5.14 ppm with *J* = 4.0 Hz in this spectrum since this amphiphile contains the maltoside head group where two glucose units are connected *via* α-glycosidic bond. A similar result was obtained from STM-E8 with the ethylene glycol linker. This agent showed a doublet peak at 4.32 ppm with *J* = 8.0 ppm in the ^{1}H NMR spectrum (Figure. 2b & S2b), supporting the exclusive formation of β-glycosidic bond. Interestingly, the β-anomeric proton (H_a) peak of STM-11 (4.79 ppm) was substantially down-fielded (Δδ = 0.47 ppm) compared to the corresponding peak of STM-E8 (4.32 ppm). SHG-G13 with six glucoside units showed rather complex NMR peaks in the anomeric region. Five doublet peaks appeared well-dispersed in the range of 4.3 to 4.6 ppm, (Figure. 2c and S2c). All coupling constants (*J*) of these anomeric peaks were 8.0 Hz, clearly indicating the successful β-linkage formation for all six glycosidic bonds. The presences of two different kinds of alcohols (primary and secondary) and an epimeric carbon in each glycerol linker are accountable for the appearance of the multiple anomeric peaks in the NMR spectrum of this agent.

Most new SIGs were soluble up to 10 wt% in water at room temperature. However, STM-10 was not soluble even at a high temperature. Because of the poor water-solubility, STM-10 was not tested in a further study. Surprisingly, the water-solubility of the STMs was increased with increasing alkyl chain length; STM-11 and STM-C12 gave the water-solubility of ~5 and ~10 wt%, respectively. The self-aggregation behaviors of the SIGs were investigated in terms of critical micellar concentrations (CMCs) and micelle size. The CMC and dynamic light scattering (DLS) data of all SIGs are summarized in Table 1. The CMCs of the individual detergents were estimated using diphenylhexatriene (DPH), a water-insoluble fluorescent dye.[42] STM-E7 and STM-E8 with relatively short alkyl chains gave the CMCs (300 and 150 μM, respectively) higher than or comparable to DDM (170 μM), but all

the SIGs except these two agents gave significantly lower CMCs than DDM, indicating their enhanced tendency to self-assemble. The CMCs of the new agents tend to decrease with increasing alkyl chain length. For instance, SHG-G14 with the longest alkyl chain gave the lowest value (8 μM), while STM-E7 with the shortest alkyl chain gave the highest value (300 μM). This result is consistent with the fact that detergent CMCs are mainly determined by the hydrophobicity of detergent lipophilic group (*i.e.*, hydrophobic effect), with a minor contribution from detergent head group.

DLS experiments were carried out to measure the size of self-assemblies formed by the SIGs. We found that the assembly sizes were substantially affected by the detergent alkyl chain length. With increasing alkyl chain length, detergent self-assembly size was substantially enlarged within each set of SIGs. For example, the aggregate sizes of the STM-Es drastically increased from 2.5 to 3.8 to 11.2 to 21.1 nm with increasing alkyl chain length from C7 to C8 to C9 to C10. In addition, detergent assembly size was profoundly influenced by the volumes of the detergent head groups (SHG-Gs *vs* STMs/STM-Es). A larger head group renders detergent molecular shape more conical, thereby decreasing the assembly size, as exemplified by a comparison of STM-12 with SHG-G12. Although these two agents have the same alkyl chain length (C12), SHG-G12 with the hexa-glucoside head group formed much smaller assemblies than STM-C12 with the tri-maltoside head group (2.7 *vs* 12.3 nm). Due to the presence of the bulky head group (i.e., hexa-glucoside), all the SHG-Gs formed smaller micelles than DDM (2.7~2.9 *vs* 3.4 nm) despite the possession of a long alkyl chain. More interestingly, STM-11/12 (9.4/12.3 nm) formed smaller micelles than STM-E10 (21.1 m) although these agents have longer alkyl chains than the latter. This result is unexpected as the head group of the STM-Es appears to be larger than that of the STMs due to the presences of the additional ethylene glycol linkers. This unexpected result suggests that the introduction of the flexible ethylene glycol linker allows the three maltose units of the STM-Es to come together, decreasing the effective volume of the head group. On the other hand, the three maltose units of the STMs appears to occupy a relatively large volume because of the lack of such a flexible linker. The homogeneity of micelles formed by each new detergent was supported by a single set of size population in its DLS profile (Figure. S3)

Detergent evaluation with membrane proteins

The protein stabilization efficacy of the new agents was first evaluated with the photosynthetic superassembly of *Rhodobacter (R.) capsulatus*, a non-sulfur, purple photosynthetic bacterium.[43] This photosynthetic superassembly comprises the photosensitive light-harvesting complex 1 (LH1) and reaction center complex (RC). The presence of multiple co-factors such as chlorophylls and carotenoids in the interior of intact complex leads to a strong absorption at λ = 875 nm that diminishes with a structural degradation of the complex. Thus, time-course structural integrity of the complex can be assessed by monitoring complex absorption at 875 nm (A_{875}) over time. The LH1-RC complex was first extracted from the membranes using 1.0 wt% DDM, followed by isolation in the same detergent via Ni^{2+}-NTA affinity chromatography. The DDM-purified complex was diluted into the individual detergent-containing buffer solutions to have CMCs + 0.2 wt % as the final detergent concentrations. Protein stability was evaluated over the course of a

30-day incubation and an incubation temperature was stepwise increased by 10 °C starting from 25 °C at every 10 days. The DDM-encapsulated LHI-RC underwent significant degradation at room temperature (Figure. 3a). After a 10-day incubation at 25 °C, the complex retained less than 40% integrity. A loss in complex integrity was accelerated with increasing incubation temperature, resulting in a complete integrity loss at the 20-day point. In contrast, all three sets of SIGs were substantially more effective than DDM at retaining complex integrity, with the best performance observed for the SHG-Gs. The SHG-Gs retained ~ 90% complex integrity after the 10-day of incubation at 25 °C (*vs* ~ 40% for DDM). Like DDM, the elevated incubation temperatures (35 and 45 °C) accelerated the complex degradation in the individual cases of the SIGs. However, SHG-G13/G14 maintained 40/60% complex integrity at the end of incubation (30-day), which is in contrast to a complete degradation of the DDM-solubilized complex even at the 20-day incubation. Overall, detergent efficacy for complex stabilization was in the order of SHG-Gs > STMs > STM-Es > DDM. The clear outperformance of the SIGs, particularly the SHG-Gs, indicates their favorable architecture for stabilization of this denaturation-sensitive protein complex.

We further tested the SIGs with the leucine transporter (LeuT) from bacteria *Aquifex aeolicus*.[44] LeuT is an ion-coupled transporter and a prokaryotic homolog of the human neurotransmitter-sodium symporter (NSS) family. After extracting using 1.0 wt% DDM, the transporter was purified in 0.05% of the same detergent. Dilution of the DDM-purified transporter into buffer solutions supplemented with the individual SIGs gave final detergent concentrations of CMCs+0.2 wt%. Transporter stability was assessed by monitoring an ability to bind tritium-labelled substrate ([^{3}H]-leucine (Leu)) via a scintillation proximity assay (SPA).[45] The substrate-binding ability was evaluated at regular intervals during a 14-day incubation at room temperature. The DDM-solubilized transporter gave high initial activity (t = 0 day) but showed a rather rapid loss in the activity over time (Figure. 3b). On the other hand, all the SIGs showed initial transporter activity lower than DDM, but all these agents except SHG-G13 were superior to this conventional detergent in maintaining the initial activity in a long-term. Detergent efficacy for LeuT stabilization appeared to be unrelated to alkyl chain length. For example, the short alkyl chain SIGs (STM-E7 and STM-E8) were comparable to the long alkyl chain detergents (STM-12 and SHG-G14) in detergent efficacy. Thanks to enhanced transporter stabilization, uses of some SIGs (e.g., STM-E7/E8/E10, STM-12, and SHG-G14) resulted in transporter activity approximately six times higher than DDM use at the end of the incubation (14-day). Interestingly, most of the new agents showed marginal or substantial increases in transporter activity over the course of the first 3-day incubation, which could be due to a slow detergent exchange during the sample dilution/incubation. The presence of three alkyl chains may hinder a fast detergent exchange from DDM to a new agent.

The promising results with LH1-RC and LeuT provoked us to further evaluate SIG performance with regards to membrane protein extraction and stabilization using the melibiose permease of *Salmonella typhimurium* ($MelB_{St}$).[46] Five SIGs (STM-E8, STM-E9, STM-C11, STM-12, and SHG-G12) were used for this experiment because these agents showed the sufficiently high water-solubility. *E. coli* membrane fragments including $MelB_{St}$ were treated with 1.5 wt% DDM or the selected SIG, followed by an incubation at a specified temperature for 90 minutes. The amounts of soluble $MelB_{St}$ under the tested

conditions were quantified by SDS-PAGE and Western blotting analysis, and expressed as percentages of the initial amount of $MelB_{St}$ present in the membranes. As shown in Figure. 4a, DDM quantitatively extracted the transporter at 0 °C. The new agents except SHG-G12 yielded 70~90% soluble $MelB_{St}$ under the same conditions, thereby being a little inferior to DDM in protein solubilization efficiency. At an elevated temperature of 45 °C, the amounts of soluble $MelB_{St}$ were increased to the level of 80~100% for all the tested new agents except SHG-G12. When the incubation temperature was further elevated to 55 °C, detergent efficacy for $MelB_{St}$ solubilization was clearly differentiated. At this high temperature, DDM gave about 10% soluble $MelB_{St}$ while STM-11 and STM-12 yielded more than 50% soluble $MelB_{St}$, indicating that these new agents were not only efficient at extracting the transporter, but were also effective at preserving the transporter in a soluble state under the conditions. None of the tested agents were successful in preserving $MelB_{St}$ solubility at 65 °C. To further evaluate detergent effectiveness for $MelB_{St}$ functionality, STM-12 was selected for a comparison with DDM. $MelB_{St}$ function was assessed by melibiose reversal of Förster resonance energy transfer (FRET) from tryptophan to 2'-(*N*-dansyl)aminoalkyl-1-thio-β-D-galactopyranoside (D^2G).[45] An active transporter efficiently binds to both galactosides (*i.e.*, D^2G (ligand) and melibiose (substrate)). Consequently, a strong florescent signal of D^2G-bound $MelB_{St}$ could be reversed by the addition of a competitive and non-fluorescent substrate (melibiose) as a ligand-substrate exchange occurs in the binding pocket. Thus, the functional state of detergent-solubilized $MelB_{St}$ can be addressed by monitoring protein fluorescence signal over the course of the sequential addition of D^2G and melibiose. The DDM-solubilized $MelB_{St}$ was well responsive to the additions of both D^2G and melibiose (Figure 4b). However, a complete loss in transporter function was observed when a less stable homologue, $MelB_{EC}$ obtained from *E. coli*, was used under the same conditions.[48] In contrast, STM-12 preserved the functionality of both MelB homologues.[48] Collectively, STM-12 was superior to DDM at maintaining MelB in the soluble and functional form.

The novel detergents continued to be evaluated with the human β_2 adrenergic receptor (β_2AR), a G-protein coupled receptor (GPCR).[49] The receptor was isolated in 0.1 wt% DDM from the membrane. For detergent exchange, the DDM-purified receptor was mixed with buffer solutions containing the individual SIGs such that final detergent concentrations became CMCs + 0.2 wt%. The ability of the receptor to bind the radioactive antagonist ([^{3}H]-dihydroalprenolol (DHA)) was utilized to directly assess receptor stability in a given detergent.[50] As for a preliminary evaluation, the initial ligand-binding ability of the receptor was measured after a 30-min detergent exchange (Figure. S4a). This result prompted us to select several new agents (STM-E7, STM-E10, STM-11, STM-12, and SHG-G14) to further evaluate detergent efficacy for long-term receptor stabilization. Receptor activity was measured at regular intervals over a three- or five-day incubation at room temperature (Figure. S4b, c). DDM showed a gradual decrease in receptor activity over time. STM-E10 and SHG-G14 were a little worse than DDM in this regard, while STM-11 and STM-12 were more or less comparable to DDM although initial receptor activity was rather low. Interestingly, a short alkyl chain detergent (STM-E7) was better than DDM at maintaining receptor activity long term. However, we could not find a new agent markedly better than DDM at stabilizing the receptor.

A few of the new detergents were selected for further comparison with lauryl maltose-neopentyl glycol (LMNG), a significantly optimized novel detergent for membrane protein study.[27] For further stability analysis with LHI-RC, we selected STM-11, STM-12, SHG-G13 and SHG-G14 and tested these agents at CMC+0.2 wt% for 10 days. Consistent with previous result,[27] LMNG was better than DDM. All the selected agents were superior to this NG-based detergent in stabilizing the complex, with a slightly better performance for the SHGs than the STMs (Figure S5a). An alternative set of novel detergents (STM-E8, STM-E10, STM-12 and SHG-G14) were tested at CMC+0.2 wt% with LeuT for a comparison with LMNG. As expected, LMNG was better than DDM at stabilizing LeuT. All tested new detergents were even more effective than LMNG (Figure S5b). Of note, no SIG is likely better than LMNG as for β_2AR stability.[25,27] These results indicate that a few new detergents including STM-12 and SHG-G14 can be used as alternatives to LMNG for membrane protein study.

This study introduced the three sets of SIGs (STMs, STM-Es and SHG-Gs) with three alkyl tails and multiple carbohydrates (three maltoses or six glucoses), respectively projecting from the scyllo-inositol core in an opposite direction. A systematic change in the head group architecture allowed us to prepare the new agents with a range of alkyl chain length (C7 ~ C14) and with a large variation in self-assembly size (2.5 ~ 21.1 nm). Because of the presence of the large head group (*i.e.*, hexa-glucoside), all the SHG-Gs formed notably small micelles compared to DDM and the other sets of SIGs (STM-Es and STMs) despite the presence of a long alkyl chain (C12, C13 or C14). In the evaluation with several membrane proteins, we found that many new agents were significantly superior to DDM for both LHI-RC and LeuT stability, while two detergents (STM-11 and STM-12) were clearly better than this conventional detergent for MelB stability. As for β_2AR stability, STM-E7 was the only one better than DDM. The best detergent for protein stability varied depending on the tested membrane proteins here. SHG-G13/G14 and STM-E8/E10 were among the best at stabilizing LHI-RC and LeuT, respectively. As for MelB and β_2AR stability, the best detergents were STM-11/12 and STM-E7, respectively. Thus, there was no single agent that effectively stabilizes all the membrane protein tested here, consistent with the general notion that there is unlikely a magic bullet for all membrane proteins. However, the systematic comparison of the three sets of SIGs implies the generally favorable property of the STMs relative to the other sets (STM-Es and SHG-Gs). Although a favorable behavior was not obviously detected for β_2AR stability, these agents, particularly STM-12, were most outstanding at stabilizing MelB and nearly comparable to the best agents for LHI-RC and LeuT stability. Of the SIGs, thus, the STMs appeared to possess the most universal property for membrane protein stabilization.

Detergent alkyl chain length is often important in determining detergent efficacy for protein stabilization. Membrane proteins have the cylindrical hydrophobic surface with a range of width of 28 to 32 Å[51] and thus detergent alkyl chain length needs to be compatible with the hydrophobic dimensions of membrane proteins for protein stability. In this context, the overall favorable behavior of STM-11/12 for protein stabilization observed here seems reasonable as their alkyl chain lengths are in the optimal range (C11 to C13). However, it is noteworthy to mention that STM-12 and SHG-G12 showed a large difference in their efficacy for membrane protein stabilization despite the possession of the same alkyl chain

length (C12). STM-12 were substantially better than SHG-G12 at stabilizing the tested membrane proteins except for LHI-RC where an opposite trend was observed; SHG-G12 was slightly better than STM-12 at stabilizing this denaturation-prone protein complex. This result suggests the presence of another important factor in determining detergent efficacy for protein stabilization. As these two agents have similar CMCs (0.01 mM for STM-12 and 0.015 mM for SHG-G12), a detergent tendency to self-aggregate is unlikely responsible for this notable difference. On the other hand, the micelles formed by these two agents significantly differ from each other in terms of size (12.3 nm for STM-12 *vs* 2.7 nm for SHG-G12). Thus, detergent micelles size could be linked to the superiority of STM-12 to SHG-G12 observed here.[52] For effective protein stabilization, the hydrophobic surfaces of membrane proteins need to be sufficiently encapsulated by detergent molecules to prevent their aggregation. Thus, a detergent (e.g., SHG-G12) that forms too small micelles would be suboptimal at stabilizing membrane proteins, particularly for aggregation-prone proteins including GPCRs. Based on this discussion, two detergent characteristics, an optimal alkyl chain length and sufficiently large micelle formation, seem to be critical factors for the universal stabilization of diverse membrane proteins. It is noteworthy that STM-11/12 possessing both characteristics was not superior to DDM for β_2AR stability. This result indicates the presence of an additional factor important for protein stabilization that is unclear at this point. The suboptimal property of STM-11/12 for β_2AR stability could be due to the presence of empty spaces within the detergent micelle interior, particularly in the hydrophobic-hydrophilic interface.[53] A mono-saccharide ring introduced here may not be large enough to effectively eliminate these empty spaces.

Conclusions

In summary, this study introduces the scyllo-inositol-cored glycoside amphiphiles (STMs, STM-Es and SHG-Gs) with multiple head and tail groups effectively segregated from each other. Distinct from conventional and other novel detergents, the monosaccharide ring in the central region endows the new detergents with modulated molecular rigidity, high hydrophobic density and effective micellar packing. These distinctive properties are likely associated with the superior stabilization efficacy of these amphiphiles, particularly for the STMs, for most of the tested proteins here. The comparative analysis of STM-12 *vs* SHG-G12 data and speculation on the suboptimal efficacy of STM-12 for β_2AR stabilization enabled us to extract the plausible detergent properties critical for membrane protein stabilization. Therefore, this study not only introduces new detergent tools for membrane protein study, but also suggests the important detergent design principles for the future development of novel detergents. An optimization via the structural modifications will further improve SIG efficacy for membrane protein solubilization and stabilization.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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REFERENCES

[1]. Wallin E and von Heijne G, Genome-wide analysis of integral membrane proteins from eubacterial, archaean, and eukaryotic organisms. Protein Sci. 1998, 7, 1029–1038. [PubMed: 9568909]

[2]. Singer SJ, Some early history of membrane molecular biology. Annu. Rev. Physiol 2004, 66, 1–27. [PubMed: 14977394]

[3]. Sanders CR and Nagy JK, Misfolding of membrane proteins in health and disease: the lady or the tiger? Curr. Opin. Struct. Biol 2000, 4, 438–442.

[4]. Cook JL, G Protein-Coupled receptors as disease targets: Emerging paradigms. Ochsner J. 2010, 1, 2–7.

[5]. Montfort RLMV and Workman P, Structure-based drug design: aiming for a perfect fit. Essays Biochem. 2017, 61, 431–437. [PubMed: 29118091]

[6]. Deisenhofer J, Epp O, Miki K, Huber R and Michel H, Structure of the protein subunits in the photosynthetic reaction centre of Rhodopseudomonas viridis at 3Å resolution. Nature 1985, 318, 618–624. [PubMed: 22439175]

[7]. https://www.wwpdb.org/

[8]. Yang Z, Wang C, Zhou Q, An J, Hildebrandt E, Aleksandrov LA, Kappes JC, DeLucas LJ, Riordan JR, Urbatsch IL, Hunt JF and Brouillette CG, Membrane protein stability can be compromised by detergent interactions with the extramembranous soluble domains. Protein Sci. 2014, 23, 769–789. [PubMed: 24652590]

[9]. Min D, Jefferson RE, Qi Y, Wang JY, Arbing MA, Im W and Bowie JU, Unfolding of a ClC chloride transporter retains memory of its evolutionary history. Nat. Chem. Biol.volume 2018, 14, pages489–496.

[10]. Cheng Y, Membrane protein structural biology in the era of single particle cryo-EM. Curr. Opin. Struct. Biol 2018, 52, 58–63. [PubMed: 30219656]

[11]. Parker JL and Newstead S, Current trends in α-helical membrane protein crystallization: An update. Protein Sci. 2012, 21, 1358–1365. [PubMed: 22811290]

[12]. Serrano-Vega MJ, Magnani F, Shibata Y and Tate CG, Conformational thermostabilization of the β1-adrenergic receptor in a detergent-resistant form. Proc. Natl. Acad. Sci. U. S. A 2008, 105, 877–882. [PubMed: 18192400]

[13]. Privé GG, Detergents for the stabilization and crystallization of membrane proteins. Methods 2007, 41, 388–397. [PubMed: 17367711]

[14]. Alguel Y, Leung J, Singh S, Rana R, Civiero L, Alves C, and Byrne B, New tools for membrane protein research. Curr. Protein Pept. Sci 2010, 11, 156–65. [PubMed: 20088775]

[15]. Ujwal R and Bowie JU, Crystallizing membrane proteins using lipidic bicelles. Methods 2011, 55, 337–341. [PubMed: 21982781]

[16]. Denisov IG and Sligar SG, Nanodiscs for structural and functional studies of membrane proteins. Nat. Struct. Mol. Biol 2016, 23, 481–486. [PubMed: 27273631]

[17]. Polidori A, Raynal S, L-A. Barret, M. Dahani, C. B-Ivolot, C. Jungas, E. Frotscher, S. Keller, C. Ebel, C. Breyton and F. Bonneté, Sparingly fluorinated maltoside-based surfactants for membrane-protein stabilization. New J. Chem 2016, 40, 5364–5378.

[18]. Popot JL, Althoff T, Bagnard D, Banères JL, Bazzacco P, Billon-Denis E, Catoire LJ, Champeil P, Charvolin D, Cocco MJ, Crémel G, Dahmane T, de la Maza LM, Ebel C, Gabel F, Giusti F, Gohon Y, Goormaghtigh E, Guittet E, Kleinschmidt JH, Kuhlbrandt W, Le Bon C, Msartinez KL, Picard M, Pucci B, Sachs JN, Tribet C, van Heijenoort C, Wien F, Zito F and Zoonens M, Amphiphols from A to Z. Annu. Rev. Biophys 2011, 40, 379–408. [PubMed: 21545287]

[19]. Dörr JM, Scheidelaar S, Koorengevel MC, Dominguez JJ, Schäfer M, Van Walree CA and Killian JA, The styrene-maleic acid copolymer: A versatile tool in membrane research. Eur. Biophys. J 2016, 45, 3–21. [PubMed: 26639665]

[20]. McGregor C-L, Chen L, Pomroy NC, Hwang P, Go S, Chakrabartty A and Privé GG, Lipopeptide detergents designed for the structural study of membrane proteins. Nat. Biotechnol 2003, 21, 171–176. [PubMed: 12524549]

[21]. Tao H, Lee SC, Moeller A, Roy RS, Siu FY, Zimmermann J, Stevens RC, Potter CS, Carragher B and Zhang Q, Engineered nanostructured β-sheet peptides protect membrane proteins. Nat. Methods 2013, 10, 759–761. [PubMed: 23817067]

[22]. McQuade DT, Quinn MA, Yu SM, Polans AS, Krebs MP and Gellman SH, Rigid amphiphiles for membrane protein manipulation. Angew. Chem., Int. Ed 2000, 39, 758–761.

[23]. Cho KH, Du Y, J Scull N, Hariharan P, Gotfryd K, Loland CJ, Guan L, Byrne B, K Kobilka B and Chae PS, Novel xylene-linked maltoside amphiphiles (XMAs) for membrane protein stabilisation. Chem. – Eur. J 2015, 21, 10008–10013. [PubMed: 26013293]

[24]. Hussain H, Du Y, Tikhonova E, Mortensen JS, Ribeiro O, Santillan C, Das M, Ehsan M, Loland CJ, Guan L, Kobilka BK, Byrne B and Chae PS, Resorcinarene-based facial glycosides: Implication of detergent flexibility on membrane-protein stability. Chem. – Eur. J 2017, 23, 6724–6729. [PubMed: 28303608]

[25]. Das M, Du Y, Ribeiro O, Hariharan P, Mortensen JS, Patra D, Skiniotis G, Loland CJ, Guan L, Kobilka BK, Byrne B and Chae PS, Conformationally preorganized diastereomeric norbornane-based maltosides for membrane protein study: Implications of detergent kink for micellar properties. J. Am. Chem. Soc 2017, 139, 3072–3081. [PubMed: 28218862]

[26]. Chae PS, Rana RR, Gotfryd K, Rasmussen SGF, Kruse AC, Cho KH, Capaldi S, Carlsson E, Kobilka BK, Loland CJ, Gether U, Banerjee S, Byrne B, Lee JK and Gellman SH, Glucose neopentyl glycol (GNG) amphiphiles for membrane protein solubilization, stabilization and crystallization. Chem. Commun 2013, 49, 2287–2289.

[27]. Chae PS, Rasmussen SGF, Rana RR, Gotfryd K, Chandra R, Goren MA, Kruse AC, Nurva S, Loland CJ, Pierre Y, Drew D, Popot JL, Picot D, Fox BG, Guan L, Gether U, Byrne B, Kobilka B and Gellman SH, Maltose-neopentyl glycol (MNG) amphiphiles for solubilization, stabilization and crystallization of membrane proteins. Nat. Methods 2010, 7, 1003–1008. [PubMed: 21037590]

[28]. Sadaf A, Capaldi S, Tikhonova E, Mortensen JS, Loland CJ, Guan L, Byrne B and Chae PS, A class of rigid linker-bearing glucosides for membrane protein structural study. Chem. Sci 2016, 7, 1933–1939. [PubMed: 27110345]

[29]. Ehsan M, Du Y, Scull NJ, Tikhonova E, Tarrasch J, Mortensen JS, Loland CJ, Skiniotis G, Guan L, Byrne B, Kobilka B and Chae PS, Highly branched penta-saccharide-bearing amphiphiles for membrane protein studies. J. Am. Chem. Soc 2016, 138, 3789–3796. [PubMed: 26966956]

[30]. Sadaf A, Du Y, Santillan C, Mortensen JS, Molist I, Seven AB, Hariharan P, Skiniotis G, Loland CJ, Kobilka BK, Guan L, Byrne B and Chae PS, Dendronic trimaltoside amphiphiles (DTMs) for membrane protein study. Chem. Sci 2017, 8, 8315–8324. [PubMed: 29619178]

[31]. Howell SC, Mittal R, Huang L, Travis B, Breyer RM and Sanders CR, CHOBIMALT: A cholesterol-based detergent. Biochemistry 2010, 49, 9572–9583 [PubMed: 20919740]

[32]. Chae PS, Rasmussen SGF, Rana RR, Gotfryd K, Kruse AC, Nurva S, Gether U, Guan L, Loland CJ, Byrne B, Kobilka BK and Gellman SH, A new class of amphiphiles bearing rigid hydrophobic groups for solubilization and stabilization of membrane proteins. Chem. – Eur. J 2012, 18, 9485–9490. [PubMed: 22730191]

[33]. Zhang Q, Ma X, Ward A, Hong W-X, Jaakola V-P, Stevens RC, Finn MG and Chang G, Designing facial amphiphiles for the stabilization of integral membrane proteins. Angew. Chem., Int. Ed 2007, 46, 7023–7025.

[34]. Rasmussen SGF, Choi HJ, Fung JJ, Pardon E, Casarosa P, Chae PS, DeVree BT, Rosenbaum DM, Thian FS, Kobilka TS, Schnapp A, Konetzki I, Sunahara RK, Gellman SH, Pautsch A, Steyaert J, Weis WI and Kobilka BK Structure of a nanobody-stabilized active state of the β2 adrenoceptor. Nature 2011, 469, 175–180. [PubMed: 21228869]

[35]. Kruse AC, Hu J, Pan AC, Arlow DH, Rosenbaum DM, Rosemond E, Green HF, Liu T, Chae PS, Dror RO, Shaw DE, Weis WI, Wess J and Kobilka BK, Structure and dynamics of the M3 muscarinic acetylcholine receptor. Nature 2012, 482, 552–556. [PubMed: 22358844]

[36]. Guo H, Bueler SA, and Rubinstein JL, Atomic model for the dimeric FO region of mitochondrial ATP synthase. Science 2017, 358, 936–940. [PubMed: 29074581]

[37]. Anderson JE, The six eclipsed sidechains in scyllo-inositol hexamethyl ether. A molecular mechanics study. J. Chem. Soc., Perkin Trans 2 1993, 0, 441–443.

[38]. Siddiqui N, Singh V, Deshmukh MM and Gurunath R, Structures, stability and hydrogen bonding in inositol conformers. Phys. Chem. Chem. Phys 2015, 17, 18514–18523. [PubMed: 26108975]

[39]. Chae PS, Bae HE and Das M, Adamantane-based amphiphiles (ADAs) for membrane protein study: Importance of a detergent hydrophobic group in membrane protein solubilization. Chem. Commun 2014, 50, 12300–12303.

[40]. Bae HE, Mortensen NJS, Ribeiro O, Du Y, Ehsan M, Kobilka BK, Loland CJ, Byrne B and Chae PS, Tandem neopentyl glycol maltosides (TNMs) for membrane protein stabilization. Chem. Commun. 2016, 52, 12104–12107.

[41]. Lee HW and Kishi Y, Synthesis of mono- and unsymmetrical bis-orthoesters of scyllo-inositol. J. Org. Chem 1985, 50, 4402–4404.

[42]. Chattopadhyay A and London E, Fluorimetric determination of critical micelle concentration avoiding interference from detergent charge. Anal. Biochem 1984, 139, 408–412. [PubMed: 6476378]

[43]. Labile PD, Kirmaier C, Udawatte CS, Hofman SJ, Holten D and Hanson DK, Quinone reduction via secondary B-branch electron transfer in mutant bacterial reaction centers. Biochemistry 2003, 42, 1718–1730. [PubMed: 12578387]

[44]. Deckert G, Warren PV, Gaasterland T, Young WG, Lenox AL, Graham DE, Overbeek R, Snead MA, Keller M, Aujay M, Huber R, Feldman RA, Short JM, Olsen GJ and Swanson RV, The complete genome of the hyperthermophilic bacterium Aquifex aeolicus. Nature 1998, 392, 353–358. [PubMed: 9537320]

[45]. Yamashita A, Singh SK, Kawate T, Jin Y and Gouaux E, Crystal structure of a bacterial homologue of Na+/Cl--dependent neurotransmitter transporters. Nature 2005, 437, 215–223. [PubMed: 16041361]

[46]. Swaminath G, Steenhuis J, Kobilka B and Lee TW, Allosteric modulation of beta2-adrenergic receptor by Zn(2+). Mol. Pharmacol 2002, 61, 65–72. [PubMed: 11752207]

[47]. Guan L, Nurva S and Ankeshwarapu SP, Mechanism of melib7ose/cation symport of the melibiose permease of Salmonella typhimurium. J. Biol. Chem 2011, 286, 6367–6374. [PubMed: 21148559]

[48]. Amin A, Hariharan P, Chae PS and Guan L, Effect of detergents on galactoside binding by melibiose permeases. Biochemistry 2015, 54, 5849–5855. [PubMed: 26352464]

[49]. Rosenbaum DM, Cherezov V, Hanson MA, Rasmussen SG, Thian FS, Kobilka TS, Choi HJ, Yao XJ, Weis WI, Stevens RC and Kobilka BK, GPCR engineering yields high-resolution structural insights into beta2-adrenergic receptor function. Science 2007, 318, 1266–1273. [PubMed: 17962519]

[50]. Mansoor SE, McHaourab HS and Farrens DL, Mapping proximity within proteins using fluorescence spectroscopy. A study of T4 lysozyme showing that tryptophan residues quench bimane fluorescence. Biochemistry 2002, 41, 2475–2484. [PubMed: 11851393]

[51]. Yao X, Parnot C, Deupi X, Ratnala VRP, Swaminath G and Farrens D, Coupling ligand structure to specific conformational switches in the beta2-adrenoceptor. Nat. Chem. Biol 2006, 2, 417–422. [PubMed: 16799554]

[52]. Columbus L, Lipfert J, Jambunathan K, Fox DA, Sim AYL, Doniach S and Lesley SA, Mixing and Matching Detergents for Membrane Protein NMR Structure Determination. J. Am. Chem. Soc 2009, 131, 7320–7326. [PubMed: 19425578]

[53]. Hong W-X, Baker KA, Ma X, Stevens RC, Yeager M and Qinghai Zhang, Design, Synthesis, and Properties of Branch-Chained Maltoside Detergents for Stabilization and Crystallization of Integral Membrane Proteins: Human Connexin 26. Langmuir 2010, 26, 8690–8696. [PubMed: 20232919]

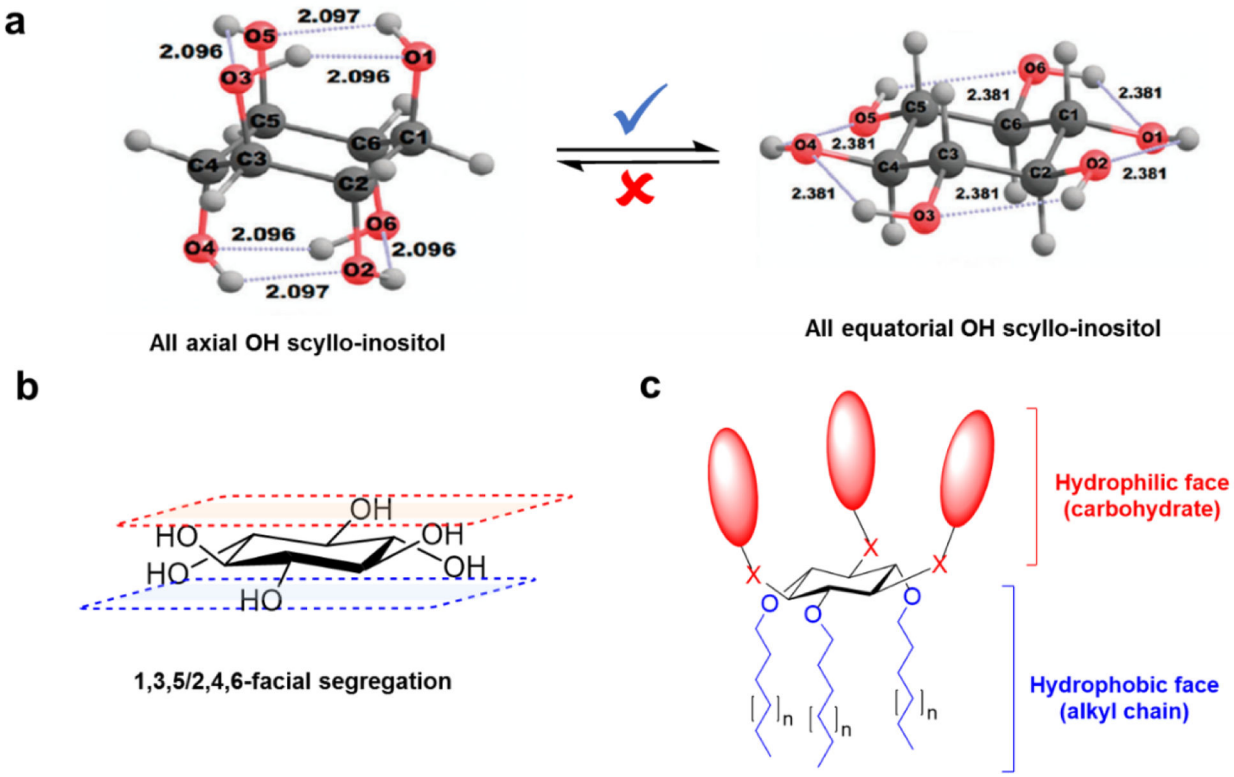


FIGURE 1.

(a) MP2/6–311+G (d,p)-optimized two conformations of scyllo-inositol unit with all hydroxyl groups in axial (left) and equatorial positions (right). Dotted lines represent intramolecular hydrogen bonding while the numbers around these dotted lines represent hydrogen bond lengths. Image was reproduced from permission of The Royal Society of Chemistry [37].(b) Schematic representation for the facial segregation of the six hydroxyl groups in scyllo-inositol. As a result of all trans-configurations of vicinal hydroxy groups, the hydroxy groups in the 1,3,5-positions are directed down, represented by a blue rectangle, while those groups in 2,4,6-positions directed up, indicated by a red rectangle. (c) Schematic chemical structures of new amphiphiles. These agents contain a scyllo-inositol unit in a central region. Three identical alkyl chains and multiple carbohydrates (glucoses or maltoses) were connected to 1,3,5- and 2,4,6-OH groups of this central unit, respectively. X represents oxygen linkage or a linker (ethylene glycol/glycerol), while red ovals do carbohydrate-based head group.

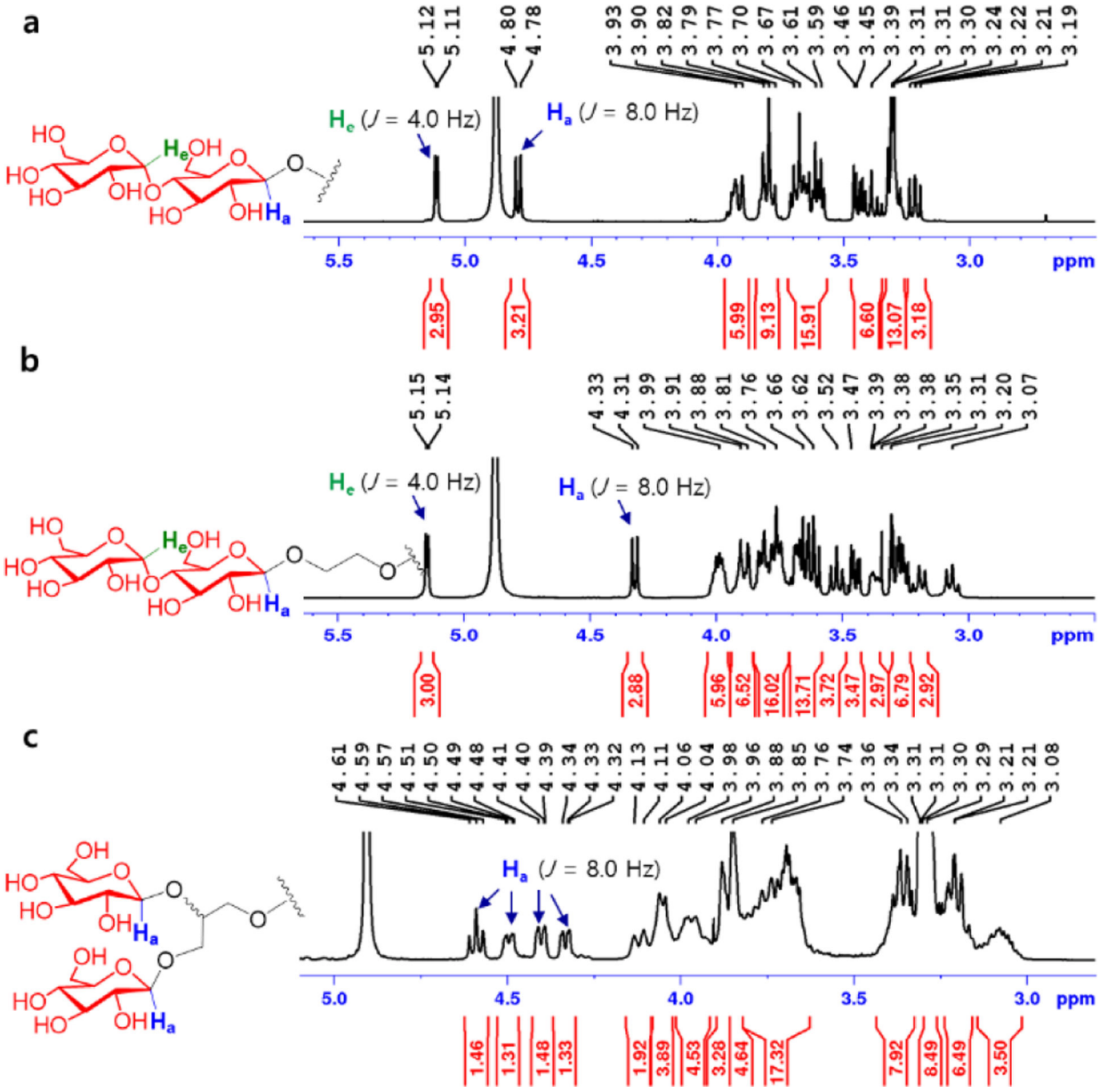


Figure. 2.

Partial ^{1}H NMR spectra of three representative SIGs: (a) STM-11, (b) STM-E8, and (c) SHG-G13. The chemical structures of the hydrophilic portions were inserted to show anomeric protons of individual amphiphiles (*left*). Both STM-C11 and STM-E8 have two kinds of anomeric protons (axial and equatorial protons (H_a and H_e)), while SHG-G13 contain only axial proton (H_a). NMR peaks corresponding to these anomeric protons were assigned in the spectra, along with their vicinal coupling constants (*J*). The chemical shifts and coupling constants detected for the anomeric protons are consistent with the stereochemistry of individual glycosidic bonds depicted in the chemical structures.

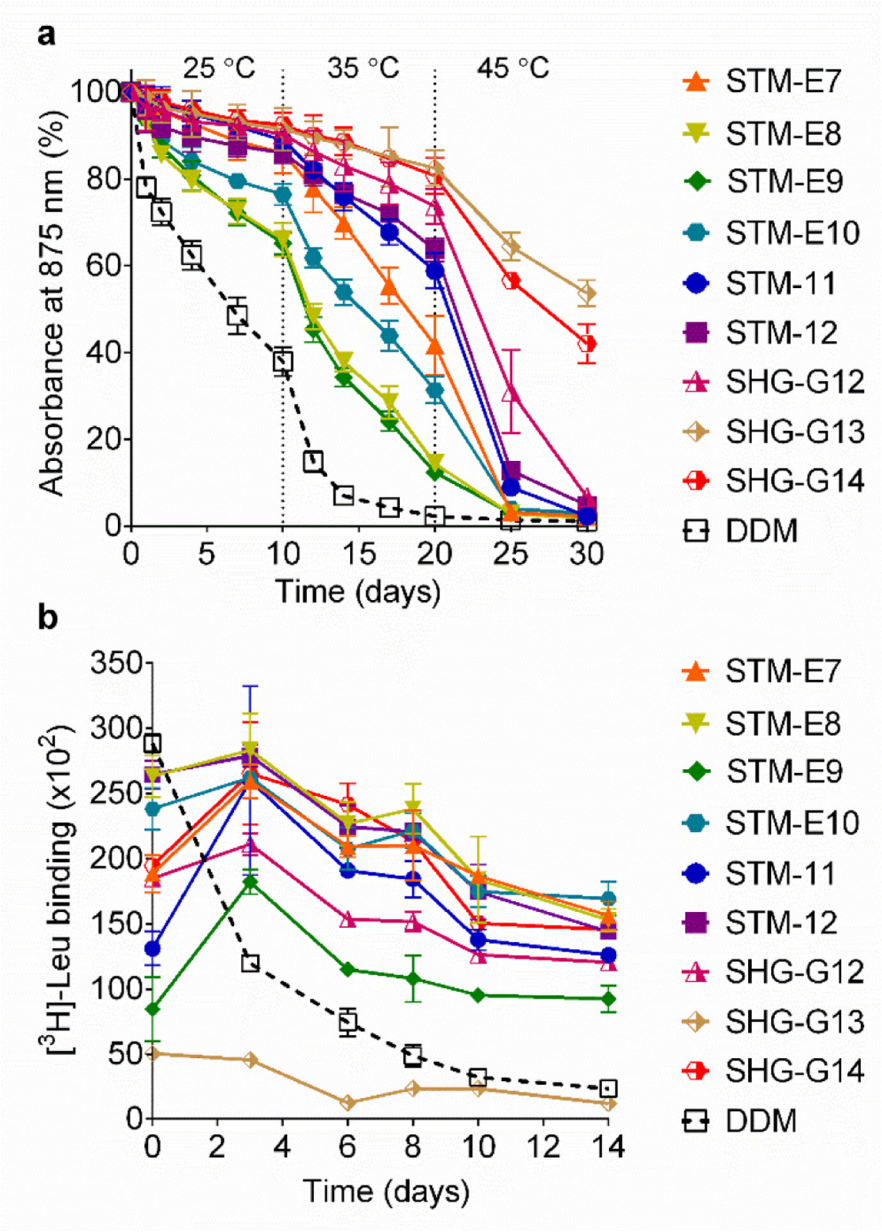


Figure. 3.

Time-course stability of (a) LHI-RC and (b) LeuT dissolved in an individual SIGs (STM-E7/E8/E9/E10, STM-11/12, or SHG-G12/G13/G14). DDM was used as a control agent. The detergents were tested at CMCs + 0.2 wt% for both proteins. LHI-RC or LeuT in purified in DDM was diluted into buffer solutions containing the individual SIGs. The sample solutions were incubated for 30 days for LHI-RC or 14 days for LeuT. As for LHI-RC, incubation temperature was stepwise increased from 25 °C to 35 °C to 45 °C at a 10-day interval, while the incubation temperature was maintained at 25 °C for LeuT. LHI-RC stability was assessed by measuring absorbance at λ = 875 nm (A_{875}) periodically over the course of the 30-day incubation. Transporter stability was assessed by monitoring the ability to bind a

radio-labelled substrate ([^{3}H]-Leucine (Leu)) at regular intervals during the incubation *via* scintillation proximity assay (SPA). Error, SEM, $n = 2$ (LHI-RC) or $n = 2–3$ (LeuT).

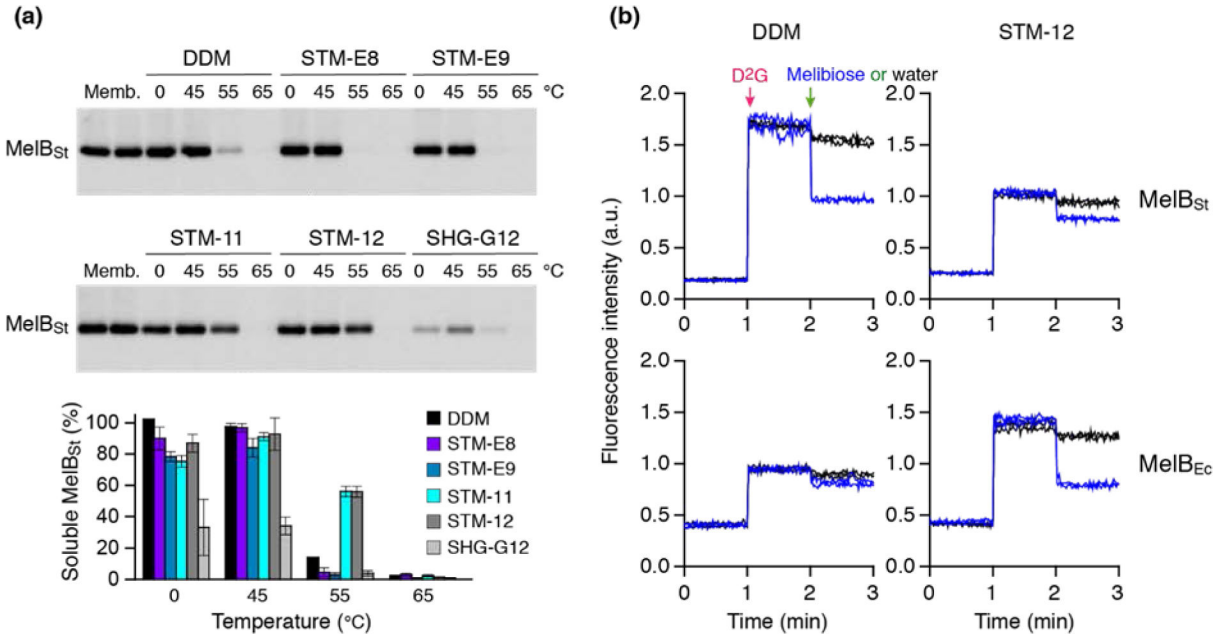
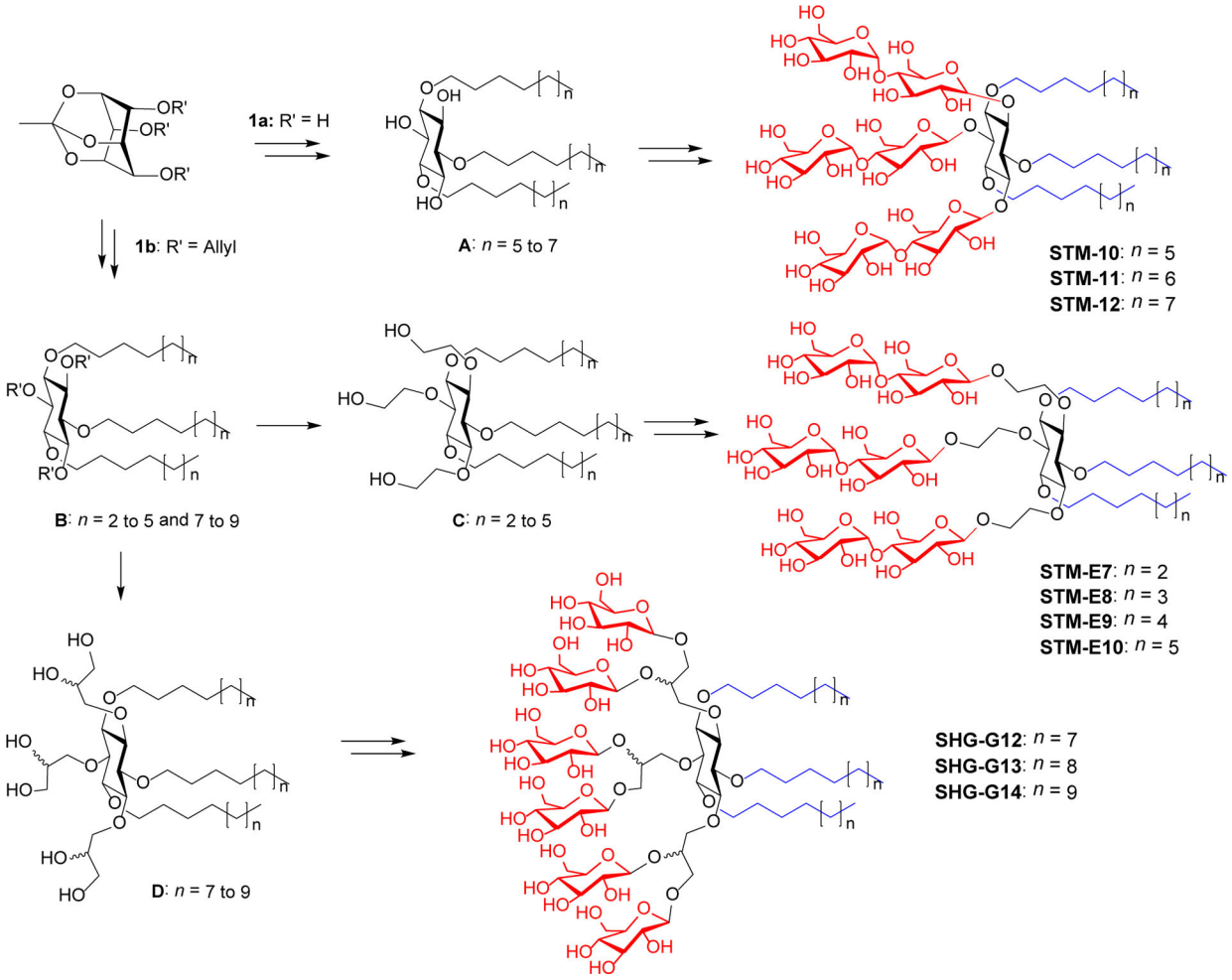


Figure. 4.

(a) Thermostability of $MelB_{St}$ solubilized in DDM, STM-E8/E9, STM-11/12, and SHG-G12. Membranes containing $MelB_{St}$ were treated with 1.5 wt% individual detergents for 90 min at four different temperatures (0, 45, 55 and 65 °C). Soluble fractions of the detergent-solubilized samples were analyzed by SDS-PAGE and Western blotting, which are summarized in the histogram. The amounts of soluble $MelB_{St}$ were expressed as percentages (%) of the total amount of the transporter initially present in the membranes (indicated in 'Memb'). (b) MelB functional study using Galactoside-binding assay. Right-side-out (RSO) membrane vesicles containing $MelB_{St}$ or $MelB_{Ec}$ were extracted with the selected detergents (DDM and STM-12). Fluorescence intensity of the detergent-solubilized MelB was monitored over time during the successive addition of D^2G and melibiose at the 1-min and 2-min time points, respectively (blue lines). For control data, water instead of melibiose was added (black lines). Data from two independent experiments are overlaid.



Scheme 1.

Synthetic scheme for preparation of three sets of SIGs (STMs, STM-Es and SHG-Gs). Compounds **1a** and **1b** were used as starting materials for the preparation of STMs and STM-Es/SHG-Gs, respectively. Compound **1a** was alkylated to give tri-ol derivatives with different alkyl chain length (**A**). A similar operation to compound **1b** following orthoester deprotection produced tri-allylated compound with different alkyl chain length (**B**). The allyl groups of these intermediates were oxidized to generate ethylene glycol/glycerol-containing intermediates (**C**/**D**). The resulting intermediates with multiple alcohol groups (**A**, **C** and **D**) were stereo-specifically glycosylated using protected maltosyl/glucosyl bromide and then subsequent deprotection gave amphipathic agents as the final products (STMs, STM-Es and SHG-Gs respectively). The wavy bonds indicate the presences of epimeric carbons in the glycerol linkers, produced in the non-stereoselective dihydroxylation step using OsO_4.

Table 1

Molecular weights (MWs), water solubility and critical micelle concentrations (CMCs) of the new agents and micelle size (R_h) (Mean ± S.D., $n = 4$) of their micelles at room temperature.

Detergent	MW[a]	CMC (mM)	CMC (wt%)	R_h(nm)[b]
STM-E7	1579.7	~ 0.300	~ 0.047	2.5 ± 0.1
STM-E8	1621.8	~ 0.150	~ 0.024	3.8 ± 0.2
STM-E9	1663.9	~ 0.035	~ 0.006	11.2 ± 0.2
STM-E10	1706.0	~ 0.010	~ 0.002	21.1 ± 0.7
STM-11	1615.9	~ 0.015	~ 0.0024	9.4 ± 0.5
STM-12	1658.0	~ 0.010	~ 0.0016	12.3 ± 0.1
SHG-G12	1880.2	~ 0.015	~ 0.0028	2.7 ± 0.2
SHG-G13	1922.3	~ 0.012	~ 0.0023	2.8 ± 0.1
SHG-G14	1964.4	~ 0.008	~ 0.0016	2.9 ± 0.1
DDM	510.6	0.170	0.0087	3.4 ± 0.1

[a]Molecular weight of detergents.

[b]Hydrodynamic radii of the micelles determined at 0.5 wt% by dynamic light scattering.