Electronic Supplementary Material (ESI) for ChemComm.

Electronic Supplementary Information

Adamantane-based Amphiphiles (ADAs) for Membrane Protein Study: Importance of Detergent Hydrophobic Group in Membrane Protein Solubilisation

Pil Seok Chae, Hyoung Eun Bae and Manabendra Das*

Dept. of Bionanotechnology, Hanyang University, Ansan, 426-791, Korea

* Corresponding author. E-mail: pchae@hanyang.ac.kr

Our hypothesis on the origin for the favorable solubilisation behavior of AD-agents

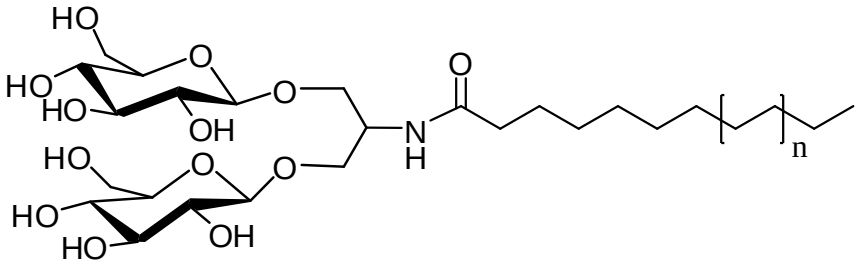
It is difficult to extract the precise reasons as to why adamantane groups are so favorable for membrane protein solubilisation. However, we believe that two characteristics of this group could be responsible for such favorable behavior. First, adamantane ring is a highly dense molecule relative to straight alkyl chains because 10 carbon units (C10) are covalently bonded to form fused ring architecture with a very small, empty interior. This high hydrophobic density could strengthen detergent interactions with membrane proteins via hydrophobic effects. In this context, AD-4 is predicted to bear the highest hydrophobic density among the AD agents owing to the absence of an alkyl chain appendage. This could explain the most favorable behavior of this agent toward its solubilisation efficiency for the superassembly. Another interesting property of AD agents responsible for their favorable solubilisation behaviors could be related to the large surface area available for close interaction with the hydrophobic segment of membrane proteins. Conventional amphiphiles with a single alkyl chain have only one terminal methyl tip available for this interaction (e.g., DDM; **Fig. S4**). Conversely, AD-1, AD-2 and AD-3 contain a methylene unit (CH_2) on the adamantane ring in addition to the methyl tip of the alkyl chains for the interaction. In the case of AD-4, which shows the highest membrane protein solubilisation efficiency, two methyl groups and three methylene units on the adamantane ring are able to interact with membrane proteins, thereby giving rise to an enhanced association with these proteins (**Fig. S4**). It is likely that the favorable behaviors of the AD agents result from the interplay of these two attributes, especially in the case of AD-4 which has both the high hydrophobic density in the lipophilic region and a large hydrophobic surface area closely interacting with membrane proteins.

Discussion about a potential relationship between detergent properties and detergent efficiency for membrane protein solubilisation

Detergent efficiency for membrane protein solubilisation is mainly determined by detergent hydrophobicity because detergent molecules interact predominantly with membrane proteins via hydrophobic interactions. Detergent hydrophobicity could also influence on detergent behaviors in micelle formation, thereby affecting detergent micelle sizes and CMC values. Detergent micelle size and CMC value tend to increase and decrease with the alkyl chain length of detergent hydrophobic group, respectively, that is associated with detergent hydrophobicity. Thus, it could be imagined to have a correlation between these detergent properties and detergent efficiency for membrane protein solubilisation. As can be seen in **Table 1S**, however, a correlation between detergent micelle size and detergent solubilisation yield turned out to be not significant. For example, a difference in micelle size between AD-2 and AD-3 is large (8 times in terms of micelle volume) but their solubilisation yields are only

slightly different (~80% *vs.* ~90%). In addition, AD-4 which forms smaller micelles than AD-3 showed higher membrane protein solubilisation yield (~100% *vs.* ~90%). In the case of conventional detergents, the smallest micelle-forming agent (LDAO) was most efficient at membrane protein solubilisation (~100%). This poor correlation between detergent micelle size and detergent efficiency for membrane protein solubilisation is mainly due to the fact that detergent micelle size is strongly affected by detergent geometry (i.e., the *relative* size of detergent head and tail groups) rather than by detergent hydrophobicity or detergent alkyl chain length. Thus, detergent micelle size is not determined by detergent tail group alone but by the *combined* effect of detergent head and tail group. A detergent CMC value is considered to be better than detergent micelle size to represent detergent hydrophobicity. Indeed, a number of membrane protein studies with various detergents have showed that a detergent with a long alkyl chain tends to give a small CMC value and increased membrane protein solubilisation yield, implying an inverse relationship between these detergent properties.[1,2] This relationship seems reasonable because detergent hydrophobicity is a main dictator for the formation of detergent micelles as well as PDCs. Note both aggregated forms, micelles and PDCs, are generated via hydrophobic interactions. Thus, there is a strong probability to find an inverse relationship between detergent CMC value and detergent efficiency for membrane protein solubilisation if we compare detergents with a same headgroup. However, this trend was neither observed amongst branched diglucoside-bearing agents (AD-1, AD-2 and two MPA-2s) nor amongst maltoside-bearing agents (AD-3, AD-4 and DDM) (**Table 1S**). AD agents gave higher membrane protein solubilisation yields than did conventional type of detergents (MPA-2s and DDM) although these agents have high CMC values relative to the latters. For instance, AD-1 with a high CMC value gave much higher membrane protein solubilisation yield than MPA-2 (C12) with a low CMC value (~ 70% *vs.* ~30%). A similar trend was observed for AD-3 and AD-4 *vs.* DDM. Such high membrane protein solubilisation efficiencies and large CMC values, two main features of AD agents, are likely to originate from the high hydrophobic density and the bulkiness of their hydrophobic groups, respectively. When we compare two maltoside-bearing AD agents (AD-3 and AD-4), a large CMC-valued AD-4 gave higher than a small CMC-valued AD-3 in terms of membrane protein solubilisation yield (~90% *vs.* ~100%). This result suggests the presence of another factor controlling membrane protein solubilization efficiency, which is here proposed to be detergent contact surface area with a membrane protein. Thus, AD-4 was efficient at solubilizing membrane protein despite having the large CMC value. In summary, individual detergent properties such as detergent micelle size, CMC value and membrane protein solubilisation efficiency are influenced by distinct detergent structural features: the *relative* size of the head and tail group for detergent micelle size, detergent hydrophobicity and the bulkiness of detergent tail group for detergent CMC value, and detergent hydrophobicity and detergent headgroup character for membrane protein solubilisation

efficiency. Therefore, a solid correlation between these detergent properties could not be found. In addition, based on the superiority of AD-4 relative to AD-3, we believe that detergent contact surface area with a membrane protein is an additional factor which is newly proposed here to explain the high efficiency of this agent for membrane protein solubilisation.



n = 0 : MPA-2 (C8) **n = 1 : MPA-2 (C10)**

n = 2 : MPA-2 (C12) **n = 3 : MPA-2 (C14)**

Figure S1. Chemical structures of previously reported monopod amphiphile-2 variants (MPA-2s) with a branched diglucoside head group. These MPA agents vary in their alkyl chain length. A previous study showed that MPA-2 with a C12 alkyl chain (MPA-2 (C12)) was most efficient among MPA-2s at the solubilisation of the superassembly, giving ~30% solubilisation yield. This result was compared with the solubilisation behaviors of AD-1 and AD-2 with the same head group in the main text.

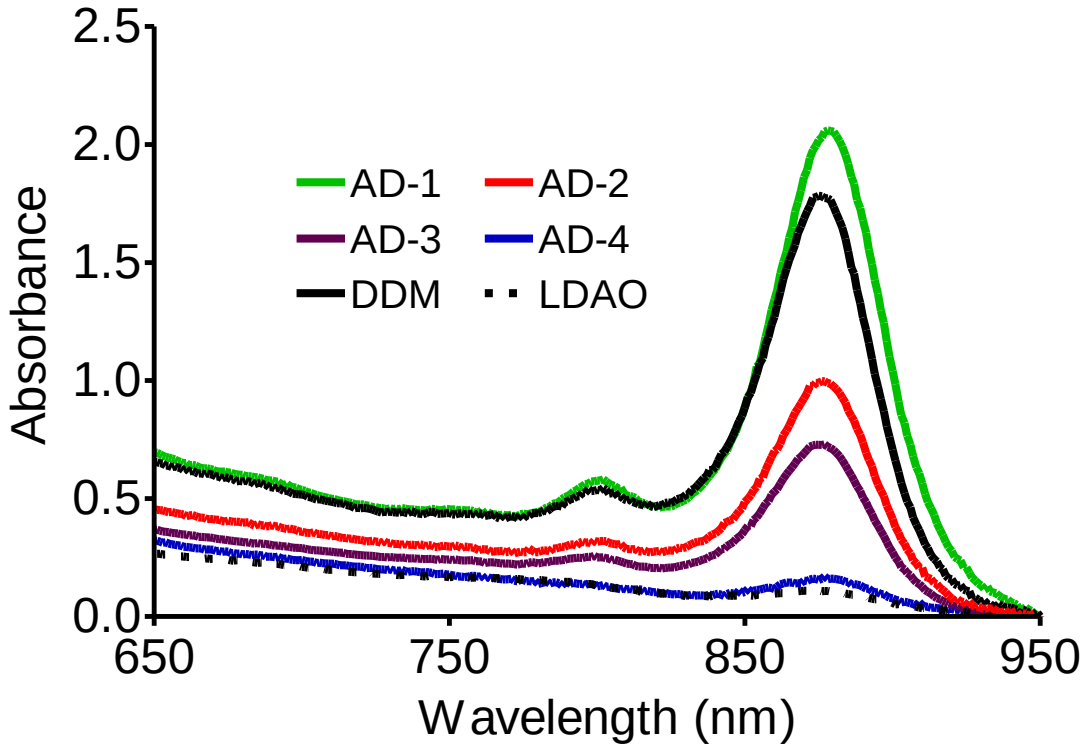
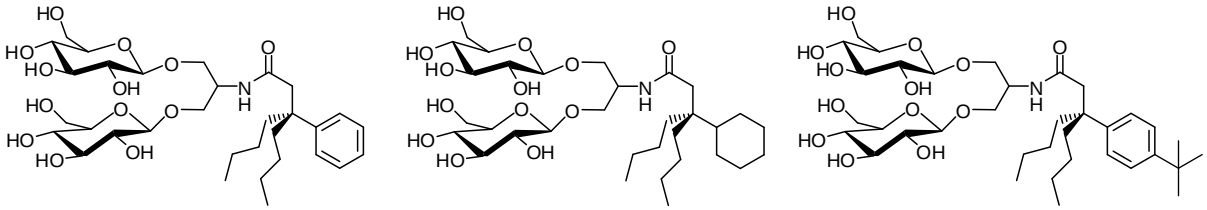


Figure S2. Spectra of protein pellet portions including insolubilized LHI-RC complexes taken after homogenization. The membrane portion that was insolubilized by the detergent treatment was obtained as a pellet via ultracentrifugation. The pellets were resuspended via homogenization prior to spectrum measurements. Protein solubilisation efficiencies of individual detergents were calculated by subtracting the protein amount remaining in the pellets from the initial amount of the superassembly.



TPA-2 **TPA-2-S** **TPA-8**

Figure S3. Chemical structures of previously reported tripod amphiphiles (TPAs) with a branched diglucoside. A previous study showed that these TPAs (TPA-2, TPA-2-S and TPA-8) extracted the LHI-RC complexes in ~50%, ~70% and ~80% yields, respectively. These solubilisation efficiencies were compared with their AD counterparts in the main text.

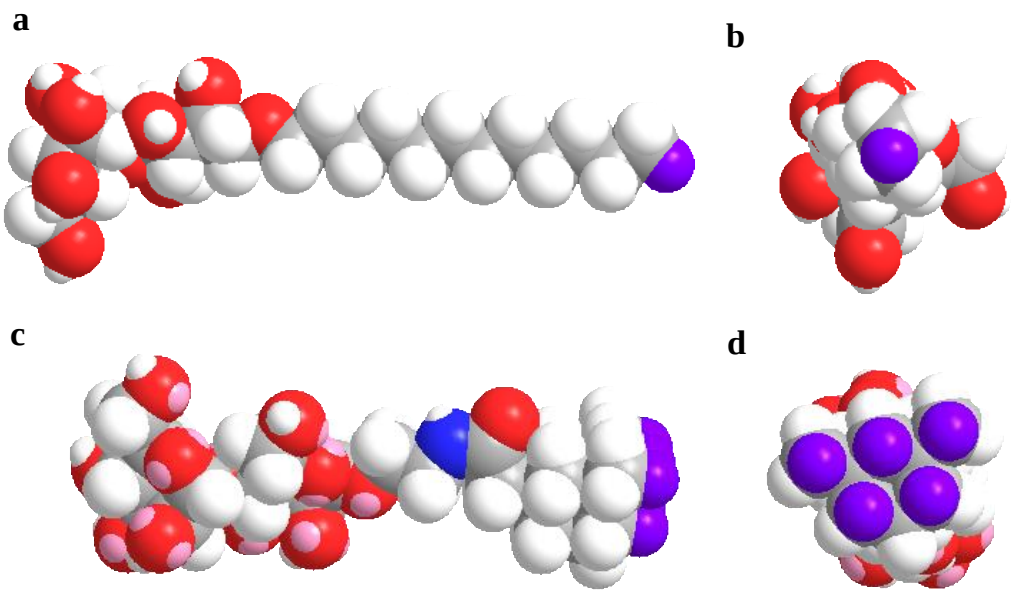


Figure S4. Space-filling models for the molecular structures of DDM and AD-4. Side and top views of DDM (**a** and **b**) and AD-4 (**c** and **d**) in terms of a molecular axis passing the longest chains are shown. The energy of each molecule was minimized via MM2 using Chem3D. Atoms are indicated by different colors (gray for carbon atoms, white for hydrogen atoms, red for oxygen atoms, blue for nitrogen atoms and pink for oxygen electron lone pairs). Hydrogen atoms closely interacting with the hydrophobic segment of membrane proteins are represented in purple. Compared to DDM, AD-4 molecule has a much larger hydrophobic surface area available for this interaction.

Table S1. Critical micelle concentrations (CMCs), hydrodynamic radii (R_h)(mean ± SD, n = 4) and protein solubilisation yields (SYs) for new AD amphiphiles (AD-1, AD-2, AD-3 and AD-4), two conventional detergents (DDM and LDAO) and previously reported monopod amphiphiles with variable chain lengths (MPA-2 (C12) and MPA-2 (C14)).

Amphiphiles	MW[a]	CMC (mM)	CMC (wt %)	R_h (nm)	SY (%)
AD-1	647.8	~6.4	~0.42	3.2 ± 0.1	~70
AD-2	675.8	~1.1	~0.074	3.5 ± 0.1	~80
AD-3	617.7	~2.6	~0.16	7.0 ± 0.0	~90
AD-4	589.7	~19	~1.1	6.6 ± 0.5	~100
DDM	510.6	~0.17	~0.0087	3.5 ± 0.1	~75
LDAO	229.4	~1.0	~0.023	2.0[b]	~100
MPA-2 (C12)[b]	611.7	~2.4	~0.15	N.D.[c]	~30
MPA-2 (C14) [b]	639.8	~0.17	~0.011	N.D.[c]	~5

[a] Molecular weight of detergents. [b] The data for these agents were obtained in a previous report.[3,4] [c]N.D. = not determined.

Protein solubilization assay

The solubilisation and purification of the *Rhodobacter (R.) capsulatus* superassembly was conducted according to a published protocol.[3] Specialized intracytoplasmic photosynthetic membranes were prepared from an engineered strain of *R. capsulatus*, which lacked the light-harvesting complex II (LHII). The solubilisation experiment began by thawing and homogenizing frozen aliquots of *R. capsulatus* membranes at room temperature. The solution was then incubated with mild agitation at 32˚C for 30 min. Subsequently, the solution was further incubated for 30 min after adding the individual detergents (50x CMC for DDM, 10x CMC for AD-2, AD-3 and LDAO, 5x CMC for AD-1 and 2x CMC for AD-4) as a solid into 1.0 mL solutions of the *R. capsulatus* superassembly. The solution was then subjected to ultracentrifugation at 310,000 *g* at 4℃ for 30 min to remove membrane debris. The spectra of solubilized supernatant and insolubilized pellet portions were taken between 650 nm and 950 nm to quantify the superassembly solubilized by the individual detergents and to assess the protein integrity. For protein purification, individual detergents-solubilized protein samples were transferred into new 1.7 mL microcentrifuge tubes containing Ni-NTA resin (pre-equilibrated and stored in an equal volume of buffer containing 10 mM Tris, pH 7.8 and 100 mM NaCl). Following one hour incubation at 4℃ for protein binding, the resins were collected and washed twice with 0.5 mL of binding buffer (a pH 7.8 Tris solution containing DDM at 1x CMC). Detergent-purified protein solutions were collected by eluting the resins three times with 0.20 mL elution buffer solutions containing 1.0 M imidazole and 1x CMC of the individual detergents (this buffer was identical to the binding buffer except for its inclusion of imidazole; the pH of each solution was readjusted to 7.8) and were then diluted with 0.4 mL binding buffer to reach 1.0 mL of solution. The UV-visible spectra of the superassembly purified in the individual detergents were taken to address the detergent protein stabilization efficacy.

Amphiphile Synthesis

General Procedure for glycosylation reactions

Method A[5]

A mixture of the alcohol to be glycosylated, AgOTf (2.4 equiv.), and 2,4,6-collidine (1.8 equiv.) in anhydrous CH_2Cl_2 (40 mL) was stirred at -45°C. A solution of perbenzoylated glucosylbromide (2.4 equiv.) in CH_2Cl_2 (40 mL) was added dropwise over 0.5 h to this suspension. Stirring was continued for 0.5 h at -45°C, and then the reaction mixture was allowed to warm to 0 °C and stirred for 1.5 h. After completion of the reaction (as detected by TLC), pyridine was added to the reaction mixture, which was then diluted with CH_2Cl_2 (40 mL) and filtered through celite. The filtrate was washed successively with 1 M aqueous $Na_2S_2O_3$ solution (40 mL), 0.1 M aqueous HCl solution (40 mL), and brine (2 x 40 mL). Then the organic layer was dried with anhydrous Na_2SO_4, and the solvent was removed by rotary evaporation. The residue was purified by silica gel column chromatography (EtOAc/hexane) to provide the desired product as a glassy solid.

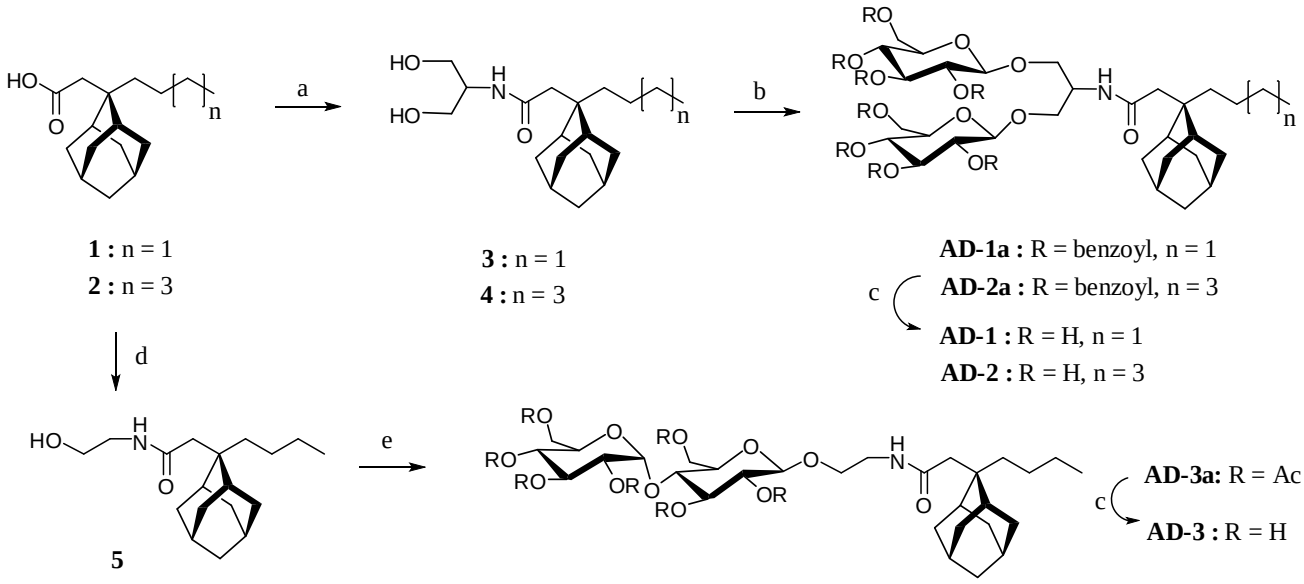
Method B[6]

$BF_3{\cdot}Et_2O$ (3.0 equiv with respect to protected maltose) was added to the 1,2-trans peracetylated maltose (1.2 equiv) and the alcohol derivatives (1.0 equiv) in dry CH_2Cl_2 under a nitrogen atmosphere at room temperature. The reaction was monitored by TLC. When the reaction did not progress further (usually 48 h), the mixture was diluted with CH_2Cl_2, and washed with saturated aqueous $NaHCO_3$ and then water. The organic layer was dried with Na_2SO_4, and concentrated by rotary evaporation. The residue was purified by silica gel column chromatography (EtOAc/ hexane) providing desired product as a glassy solid.

General procedure for de-O-acetylations or de-O-benzoylations[5]

O-protected compounds were dissolved in MeOH and treated with the required amount of a methanolic solution of 0.5 M NaOMe such that the final concentration of NaOMe was 0.05 M. The reaction mixture was stirred for 6 h at RT, and then neutralized with Amberlite IR-120 resin (H^+ form). The resin was removed by filtration and washed with MeOH, and the solvent was removed from the combined filtrate *in vacuo*. The residue was purified by silica gel column chromatography (eluting with MeOH/CH_2Cl_2). Further purification was achieved by recrystallization from CH_2Cl_2/MeOH/diethyl ether, to provide the fully de-*O*-protected product as a white solid.

Supplementary scheme 1



(a) serinol, EDC • HCl, HOBt, DMF, room temperature; (b) perbenzoylated glucosylbromide, AgOTf, CH_2Cl_2, -45°C → room temperature, 3 hr; (c) NaOMe, MeOH, room temperature, 4 hr; (d) ethanolamine, EDC • HCl, HOBt, DMF, room temperature; (e) 1,3-trans-peracetylated maltose (1.2 equiv), $BF_3{\cdot}Et_2O$, CH_2Cl_2, room temperature

Compound **1** was prepared according to a literature protocol.[7] **^{1}H NMR** (300 MHz, $CDCl_3$): δ 2.61 (s, 2H), 2.14-1.99 (m, 4H), 1.91-1.78 (m, 2H), 1.76-1.47 (m, 10H), 1.37-1.16 (m, 4H), 0.96-0.84 (m, 3H); **^{13}C NMR** (75 MHz, $CDCl_3$): δ 179.7, 41.0, 40.0, 39.0, 33.9, 33.7, 33.4, 32.9, 28.2, 27.8, 24.7, 23.7, 14.3; **HRMS (ESI)**: calcd. for $C_{16}H_{26}O_2[M-H]^+$ 249.186, found 249.186.

Compound **2** was also prepared according to a previous literature protocol.[4] **^{1}H NMR** (300 MHz, $CDCl_3$): δ 2.61 (s, 2H), 2.15-2.01 (m, 4H), 1.91-1.79 (m, 2H), 1.76-1.49 (m, 10H), 1.38-1.15 (m, 8H), 0.94-0.83 (m, 3H); **^{13}C NMR** (75 MHz, $CDCl_3$): δ 179.7, 41.0, 40.4, 39.9, 38.9, 34.0, 33.9, 33.4, 32.1, 30.4, 28.2, 28.0, 27.8, 22.9, 22.4, 14.3; **HRMS (ESI)**: calcd. for $C_{18}H_{30}O_2[M+NH_4]^+$ 296.2585, found 296.2589.

Compound **3** and **4**

Carboxylic acid (**1** or **2**, 5.0 mmol), serinol (5.5 mmol), and 1-hydroxybenzotriazole monohydrate (HOBt) (0.79 g, 5.9 mmol) was dissolved in anhydrous DMF (30 mL). 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide hydrochloride (EDC • HC1) (1.2 g, 5.9 mmol) was added in small portions at 0℃ and the resulting solution was stirred at room temperature for 24 h. The solution was taken up with ethylacetate (3 x 100 mL) and washed successively with a 1 M aqueous $NaHCO_3$ solution (100 mL), a 0.1 M aqueous HCl solution (100 mL), and brine (2 x 100 mL). Then, the organic layer was dried with Na_2SO_4 and the solvents were removed by rotary evaporation. The residue was purified by silica column chromatography (EtOAc/Hexane) to afford each product as a white solid.

Compound **3** (94% yield); **^{1}H NMR** (300 MHz, $CDCl_3$): 6.37 (d, *J* = 7.2 Hz, 1H), 3.91-3.80 (m, 1H), 3.80-3.69 (m, 2H), 3.67-3.54 (m, 2H), 2.52-2.46 (m, 2H), 2.18 (s, 2H), 2.13-2.00 (m, 4H), 1.92-1.78

(m, 2H), 1.75-1.47 (m, 10H), 1.38-1.13 (m, 4H), 0.92 (t, *J* = 7.0 Hz, 3H); **^{13}C NMR** (75 MHz, $CDCl_3$): δ 173.7, 62.4, 62.3, 52.5, 40.9, 40.8, 39.9, 34.0, 33.4, 33.3, 32.9, 28.2, 27.8, 25.0, 23.8, 14.4; **HRMS (ESI)**: calcd. for $C_{19}H_{33}NO_3[M+Na]^+$ 346.2353, found 346.2347.
Compound **4** (92% yield); **^{1}H NMR** (300 MHz, $CDCl_3$): 5.90 (d, *J* = 7.6 Hz, 1H), 3.88-3.76 (m, 1H), 3.76-3.67 (m, 2H), 3.63-3.50 (m, 2H), 3.00-2.94 (m, 2H), 2.47 (s, 2H), 2.14-1.99 (m, 4H), 1.91-1.79 (m, 2H), 1.75-1.49 (m, 10H), 1.38-1.15 (m, 8H), 0.88 (t, *J* = 7.0 Hz, 3H); **^{13}C NMR** (75 MHz, $CDCl_3$): δ 173.8, 61.6, 52.5, 52.4, 40.9, 40.7, 39.8, 34.0, 33.7, 33.3, 32.8, 32.1, 30.4, 28.1, 27.7, 22.9, 22.7, 14.2; **HRMS (ESI)**: calcd. for $C_{21}H_{37}NO_3[M+H]^+$ 352.2847, found 352.2846.

AD-1a and **AD-2a** were synthesized from diol **3** and **4,** respectively, according to the general procedure for glycosylation (*method A*). **AD-1a** (90% yield); **^{1}H NMR** (300 MHz, $CDCl_3$):): δ 8.20-8.07 (m, 4H), 8.05-7.97 (m, 4H), 7.96-7.87 (m, 4H), 7.87-7.77 (m, 4H), 7.76-7.62 (m, 6H), 7.61-7.48 (m, 4H), 7.48-7.35 (m, 10H), 7.35-7.23 (m, 4H), 5.67-5.55 (m, 3H), 5.55-5.43 (m, 2H), 5.41-5.30 (m, 2H), 4.55-4.40 (m, 2H), 4.38-4.21 (m, 3H), 3.86-3.68 (m, 4H), 3.46-3.37 (m, 1H), 3.37-3.25 (m, 2H), 2.96 (t, *J* = 9.6 Hz, 1H), 2.34 (s, 2H), 2.09-1.93 (m, 3H), 1.89-1.73 (m, 3H), 1.72-1.44 (m, 10H), 1.32-1.07 (m, 4H), 0.90 (t, *J* = 7.0 Hz, 3H); **^{13}C NMR** (75 MHz, $CDCl_3$): δ 171.8, 166.3, 166.2, 166.0, 165.9, 165.3, 165.2, 165.1, 164.9, 134.0, 133.9, 133.7, 133.6, 133.5, 133.4, 133.3, 130.2, 129.9, 129.8, 129.7, 129.4, 129.1, 129.0, 128.7, 128.6, 101.6, 101.5, 72.6, 72.4, 72.1, 72.0, 69.6, 68.0, 67.0, 63.1, 60.6, 47.1, 40.8, 39.9, 34.0, 33.4, 32.9, 28.2, 27.8, 25.9, 23.7, 21.12, 14.5, 14.4; **MS (MALDI-TOF)**: calcd. for $C_{87}H_{85}NO_{21}[M+Na]^+$ 1502.6, found 1502.9

AD-2a (91% yield); **^{1}H NMR** (300 MHz, $CDCl_3$): δ 8.20-8.08 (m, 4H), 8.06-7.97 (m, 4H), 7.96-7.88 (m, 4H), 7.88-7.79 (m, 4H), 7.76-7.61 (m, 6H), 7.61-7.48 (m, 4H), 7.48-7.34 (m, 10H), 7.34-7.21 (m, 4H), 5.67-5.55 (m, 3H), 5.55-5.41 (m, 2H), 5.42-5.30 (m, 2H), 4.56-4.40 (m, 2H), 4.40-4.20 (m, 3H), 3.90-3.71 (m, 4H), 3.47-3.39 (m, 1H), 3.39-3.26 (m, 2H), 2.98 (t, *J* = 9.6 Hz, 1H), 2.33 (t, *J* = 9.2 Hz, 2H), 2.10-1.93 (m, 4H), 1.89-1.74 (m, 2H), 1.74-1.44 (m, 10H), 1.38-1.06 (m, 8H), 0.87 (t, *J* = 7.0 Hz, 3H); **^{13}C NMR** (75 MHz, $CDCl_3$): δ 171.8, 166.3, 166.2, 166.0, 165.9, 165.3, 165.2, 165.1, 164.9, 134.0, 133.9, 133.7, 133.6, 133.5, 133.3, 130.2, 129.9, 129.8, 129.7, 129.4, 129.1, 129.0, 128.7, 128.6, 128.5, 101.6, 101.5, 72.6, 72.5, 72.2, 72.0, 69.7, 68.0, 67.1, 63.1, 47.1, 40.8, 40.7, 39.9, 34.0, 33.9, 33.7, 33.4, 32.9, 32.2, 30.4, 28.2, 27.8, 23.0, 22.9, 22.8, 14.4; **MS (MALDI-TOF)**: calcd. for $C_{89}H_{89}NO_{21}[M+Na]^+$ 1530.6, found 1530.5

AD-1 was synthesized from **AD-1a** in 95% yield according to the general procedure for de-*O*-benzoylation. **^{1}H NMR** (300 MHz, CD_3OD): δ 4.39-4.24 (m, 3H), 4.04-3.95 (m, 1H), 3.95-3.79 (m, 4H), 3.74-3.60 (m, 3H), 3.44-3.16 (m, 8H), 2.55 (s, 2H), 2.17-2.09 (m, 4H), 1.93-1.53 (m, 12H), 1.44-1.24 (m, 4H), 0.98 (t, *J* = 7.0 Hz, 3H); **^{13}C NMR** (75 MHz, CD_3OD): δ 175.4, 105.1, 104.9, 78.2, 78.1, 75.3, 71.9, 71.8, 69.9, 67.0, 63.0, 42.0, 41.2, 41.1, 35.4, 35.3, 34.5, 34.4, 34.0, 29.7, 29.3, 25.9, 24.9, 15.6, 14.9; **HRMS (ESI)**: calcd. for $C_{31}H_{53}NO_{13}[M+Na]^+$ 670.3410, found 670.3406.

AD-2 was synthesized from **AD-2a** almost quantitatively according to the general procedure for de-*O*-benzoylation. **^{1}H NMR** (300 MHz, CD_3OD): δ 4.35-4.21 (m, 3H), 4.01-3.92 (m, 1H), 3.92-3.75 (m, 4H), 3.71-3.55 (m, 3H), 3.41-3.11 (m, 8H), 2.51 (s, 2H), 2.24-2.05 (m, 4H), 1.88-1.50 (m, 12H), 1.39-1.18 (m, 8H), 0.92 (t, *J* = 7.0 Hz, 3H); **^{13}C NMR** (75 MHz, CD_3OD): δ 175.3, 105.1, 104.9, 78.2, 78.1, 75.2, 71.9, 71.8, 69.8, 63.0, 62.9, 42.0, 41.2, 41.1, 35.4, 35.3, 34.8, 34.4, 34.0, 33.4, 31.7, 29.6, 29.3, 24.0, 23.7, 14.7; **MS (MALDI-TOF)**: calcd. for $C_{33}H_{57}NO_{13}[M+Na]^+$ 698.3723, found 698.3698.

Compound **5**

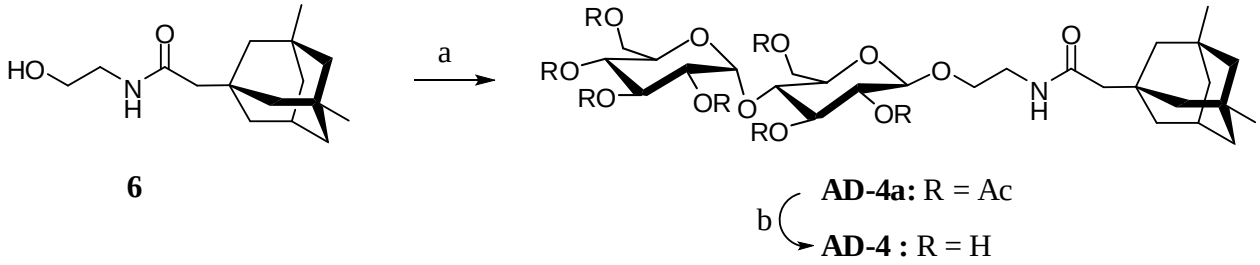
Carboxylic acid (**1**, 5.0 mmol), ethanolamine (5.5 mmol), and 1-hydroxybenzotriazole monohydrate (HOBt) (0.79 g, 5.9 mmol) was dissolved in anhydrous DMF (30 mL). 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide hydrochloride (EDC • HC1) (1.2 g, 5.9 mmol) was added in small portions at 0℃ and the resulting solution was stirred at room temperature for 24 h. The solution was taken up with ethylacetate (3 x 100 mL) and washed successively with a 1 M aqueous $NaHCO_3$ solution (100 mL), a 0.1 M aqueous HCl solution (100 mL), and brine (2 x 100 mL). Then, the organic layer was dried with Na_2SO_4 and the solvents were removed by rotary evaporation. The residue was purified by silica column chromatography (EtOAc/Hexane) to afford the product in 92% yield as a white solid. **^{1}H NMR** (300 MHz, $CDCl_3$): 5.90 (s, 1H), 3.71 (q, *J* = 5.3 Hz, 2H), 3.40 (q, *J* = 5.5 Hz, 2H), 2.92 (t, *J* = 5.1 Hz, 1H), 2.47 (s, 2H), 2.14-2.01 (m, 4H), 1.93-1.79 (m, 2H), 1.76-1.50 (m, 10H), 1.40-1.15 (m, 4H), 0.92 (s, *J* = 7.1 Hz, 3H), 0.68 (s, 3H); **^{13}C NMR** (75 MHz, $CDCl_3$): δ 174.1, 63.1, 42.7, 46.9, 39.9, 34.1, 33.5, 33.4, 32.9, 28.2, 27.8, 25.1, 23.8, 14.4; **HRMS (ESI)**: calcd. for $C_{18}H_{31}NO_2[M+Na]^+$ 316.2247, found 316.2248.

AD-3a was synthesized from alcohol derivative **5** in 83% yield according to according to according to the general procedure for glycosylation (*method B*). **^{1}H NMR** (300 MHz, $CDCl_3$): δ 5.82 (br s, 1H), 5.42 (d, *J* = 4.2 Hz, 1H), 5.36 (t, *J* = 9.8 Hz, 1H), 5.26 (t, *J* = 9.8 Hz, 1H), 5.06 (t, *J* = 9.0 Hz, 1H), 4.90-4.76 (m, 2H), 4.57-4.48 (m, 2H), 4.31-4.27 (m, 2H), 4.10-4.02 (m, 1H), 4.02-3.92 (m, 2H), 3.84-3.75 (m, 1H), 3.74-3.64(m, 2H), 3.52-3.32 (m, 2H), 2.43 (s, 3H), 2.14 (s, 3H), 2.11 (s, 3H), 2.08-1.98 (m, 16H), 1.91-1.78 (m, 2H), 1.76-1.46 (m, 10H), 1.38-1.14 (m, 4H), 0.92 (t, *J* = 7.0 Hz, 3H); **^{13}C NMR** (75 MHz, $CDCl_3$): δ 172.5, 170. 7, 170.5, 170.3, 170.1, 169.8, 169.6, 100.7, 95.8, 75.4, 72.9, 72.5, 72.3, 70.2, 69.6, 69.5, 68.7, 68.2, 62.9, 61.7, 60.5, 40.8, 40.7, 39.9, 39.1, 34.0, 33.4, 33.3, 32.9, 28.2, 27.8, 25.0, 23.7, 21.2, 21.0, 20.9, 20.8, 20.7, 14.4; **HRMS (ESI)**: calcd. for $C_{44}H_{65}NO_{19}[M+Na]^+$ 934.4043, found 934.4050.

AD-3 was synthesized from **AD-3a** in 94% yield according to the general procedure for de-*O*-acetylation. **^{1}H NMR** (300 MHz, CD_3OD): δ 5.15 (d, *J* = 3.7 Hz, 1H), 4.29 (d, *J* = 7.7 Hz, 1H), 3.98-3.76 (m, 4H), 3.76-3.56 (m, 5H), 3.56-3.34 (m, 5H), 3.34-3.17 (m, 2H), 2.49 (s, 2H), 2.27-2.04 (m,

4H), 1.92-1.69 (m, 6H), 1.69-1.48 (m, 6H), 1.42-1.20 (m, 4H), 0.93 (t, *J* = 7.0 Hz, 3H); **^{13}C NMR** (75 MHz, CD_3OD): δ 175.5, 104.6, 103.1, 81.5, 75.2, 74.9, 74.8, 74.3, 71.7, 69.8, 62.9, 62.4, 42.0, 41.3, 41.1, 40.5, 35.4, 34.5, 34.4, 34.0, 29.6, 29.3, 25.9, 24.8, 14.7; **HRMS (ESI)**: calcd. for $C_{30}H_{51}NO_{12}[M+Na]^+$ 640.3304, found 640.3300.

Supplementary scheme 2



(a) 1,3-trans-peracetylated maltose (1.2 equiv.), $BF_3{\cdot}Et_2O$, CH_2Cl_2, room temperature; (b) NaOMe, MeOH, room temperature, 4 hr.

AD-4a was synthesized from alcohol derivative **6** in 85% yield according to according to according to the general procedure for glycosylation (*method B*). **^{1}H NMR** (300 MHz, $CDCl_3$): δ 5.84 (br s, 1H), 5.41 (d, *J* = 4.1 Hz, 1H), 5.36 (t, *J* = 9.7 Hz, 1H), 5.26 (t, *J* = 9.5 Hz, 1H), 5.06 (t, *J* = 10.0 Hz, 1H), 4.90-4.76 (m, 2H), 4.58-4.48 (m, 2H), 4.31-4.17 (m, 2H), 4.10-3.91 (m, 3H), 3.86-3.76 (m, 1H), 3.75-3.63 (m, 2H), 3.54-3.32 (m, 2H), 2.15 (s, 3H), 2.11 (s, 3H), 2.07-1.98 (m, 15H), 1.96-1.91 (m, 3H), 1.47-1.40 (m, 2H), 1.33-1.28 (m, 4H), 1.28-1.14 (m, 4H), 1.13-1.01 (m, 2H), 0.81 (s, 6H); **^{13}C NMR** (75 MHz, $CDCl_3$): δ 171.3, 170.7, 170.6, 170.3, 170.1, 169.9, 169.6, 100.7, 95.8, 75.4, 72.9, 72.6, 72.4, 70.2, 69.6, 69.5, 68.7, 68.2, 62.8, 61.7, 51.1, 51.0, 49.0, 43.2, 41.3, 39.2, 34.5, 31.5, 30.7, 29.9, 21.0, 20.9, 20.8, 20.7; **HRMS (ESI)**: calcd. for $C_{42}H_{61}NO_{19}[M+Na]^+$ 906.3730, found 906.3731.

AD-4 was synthesized from **AD-4a** in 91% yield according to the general procedure for de-*O*-acetylation. **^{1}H** (300 MHz, CD_3OD): δ 5.16 (d, *J* = 3.7 Hz, 1H), 4.31 (d, *J* = 7.7 Hz, 1H), 3.99-3.74 (m, 4H), 3.74-3.57 (m, 5H), 3.55-3.33 (m, 5H), 3.33-3.18 (m, 2H), 2.10-2.02 (m, 1H), 2.00 (s, 2H), 1.51-1.42 (m, 2H), 1.40-1.16 (m, 8H), 1.15-1.01 (m, 2H), 0.82 (s, 6H); **^{13}C NMR** (75 MHz, CD_3OD): δ 175.5, 104.6, 103.1, 81.5, 75.2, 74.9, 74.8, 74.3, 71.7, 69.8, 62.9, 62.4, 42.0, 41.3, 41.1, 40.5, 35.4, 34.5, 34.4, 34.0, 29.6, 29.3, 25.9, 24.8, 14.7; **HRMS (ESI)**: calcd. for $C_{28}H_{47}NO_{12}[M+Na]^+$ 612.2991, found 612.2996.

References

1. K. H. Cho, H. E. Bae, M. Das, S. H. Gellman and P. S. Chae, *Chem. Asian J.* 2014, **9**, 632.
2. P. S. Chae, K. H. Cho, M. J. Wander, H. E. Bae, S. H. Gellman and P. D. Labile, *Biochim. Biophys. Acta* 2014, **1838**, 278

3. P. S. Chae, M. J.Wander, A. P. Bowling, P. D. Laible and S. H. Gellman, *ChemBioChem* 2008, **9**, 1706-1709.
4. J. N. Israelachvili, D. J. Mitchell and B. W. Ninham, *J. Chem. Soc., Faraday Trans. 2* 1976, **72**, 1525–1568.
5. P. R. Ashton, S. E. Boyd, C. L. Brown, N. Jayaraman, S. A. Nepogodiev and J. F. Stoddart, *Chem.-Eur. J.* 1996, **2**, 1115-1128.
6. L. A. Salvador, M. Elofsson and J. Kihlberg, *Tetrahdron* 1995, **51**, 5643-5656.
7. S. M. Yu, D. T. McQuade, M. A. Quinn, C. P. R. Hackenberger, M. P. Krebs, A. S. Polans and S. H. Gellman, *Protein Sci*., 2000, **9**, 2518–2527.