Glyco-SAMs as Glycocalyx Mimetics: Synthesis of L-Fucose- and D-Mannose-Terminated Building Blocks

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In the course of a project on the supramolecular functions of the glycocalyx present on eukaryotic cell surfaces, it has been our goal to prepare spacer glycosides and cluster glycosides that are suitable for the formation of self-assembled monolayers (SAMs) on gold. We have selected amino-functionalized D-mannose and L-fucose derivatives for peptide coupling to thio-functionalised alkane and alkane-oligoethylene glycol spacers such as **12** and **15**. Thus, a variety of thio-spacered glycosides (**16**–**19**) and cluster glycosides (**23**, **24**, **26**, **28**, and **29**) were synthesized, which can be assembled on gold wafers to serve as glycocalyx mimetics.

Introduction

All eukaryotic cells are covered by a thick layer composed of complex carbohydrates. This sugar layer is called the "glycocalyx" and possesses a thickness of up to 100 nm and more. The elucidation of the biological functions of the glycocalyx has opened up the field of glycobiology.[1] Current investigations are concentrated on the analysis of the molecular recognition and interactions of protein receptors, like the lectins[2] and selectins,[3] and their carbohydrate ligands. Such carbohydrate−protein interactions[4] are characterized by dissociation constants (K_D) in the millimolar or high micromolar range and it has been understood that it is multivalency of the respective molecular interactions[5] on a supramolecular level which leads to a biologically effective signal.[6] Numerous target-designed carbohydrate ligands, glycomimetics and multivalent neoglycoconjugates have been synthesized and investigated in binding studies of various kinds.[7] However, it has remained difficult to address the supramolecular chemistry that is most likely involved in the biology of a nano-sized array of molecules such as in the glycocalyx.[8] As it is our goal to check out the biological functions of glycocalyx carbohydrates beyond the investigation of more or less specific interactions of single molecules, we have started a project to utilize the phenomenon of self-assembly of thio-functionalised molecules on gold surfaces to mimic the expansion of a cell surface glycocalyx at least in two dimensions. Self-assembled monolayers (SAMs) were first introduced by Whitesides et al. in 1988[9] and have found wide applications since then,[10] especially in surface plasmon resonance.[11]

The target molecules of our study consist of a thio- or thioacetate-functionalised linear spacer to allow formation of a stable S−Au bond upon SAM formation on a gold wafer, and a carbohydrate part as the terminal moiety. The design of these building blocks, which are suitable for the formation of glyco-functionalized SAMs, is based on peptide-coupling of the various components (Figure 1). A hexaethylene glycol (EG_6) moiety is included to eliminate nonspecific binding of proteins.[12] To allow a facile access to more complex glyco-SAMs, we decided to use the well-established cluster glycosides[13] to serve as oligosaccharide mimetics, in addition to simple monosaccharides to terminate the S-functionalised spacers. From the armada of biologically important carbohydrates, we have selected D-mannose and L-fucose for our study, firstly because we are especially interested in mannose-specific proteins such as Concanavaline A[14] and type 1 fimbriae of bacteria,[15] and secondly because L-fucose is an important glycocalyx component associated with many sugar-related diseases and a monosaccharide with a demanding chemistry due to its labile glycosidic bond.

Results and Discussion

To obtain sugar-modified building blocks suitable for the formation of self-assembled monolayers on gold wafers, we planned to attach monovalent saccharides and eventually trivalent cluster glycosides to a thio-functionalized spacer by peptide coupling. Thus, the two essential moieties of such molecules — the saccharide and the spacer part — had to be functionalized with an amino function, which was

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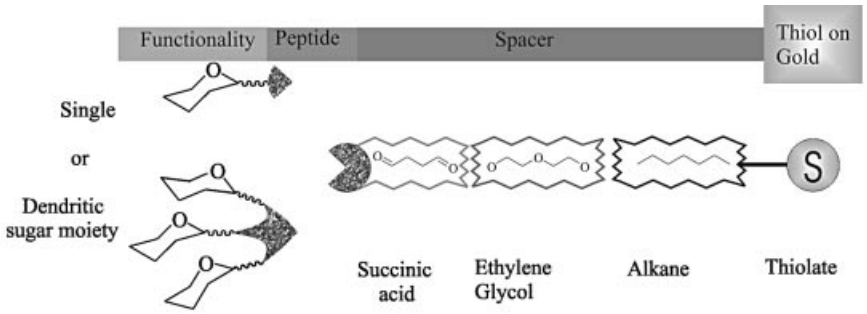


Figure 1. Architecture of thio-spacered cluster glycosides and monosaccharides, respectively, for the construction of "glyco-SAMs"

chosen for the sugar portion, and a carboxy group, which was selected as the spacer terminus. Our first task was to prepare the amino-functionalized glycosides of the mannose and fucose series — the β-fucosides **5** and **6** and the α-mannoside **10**.

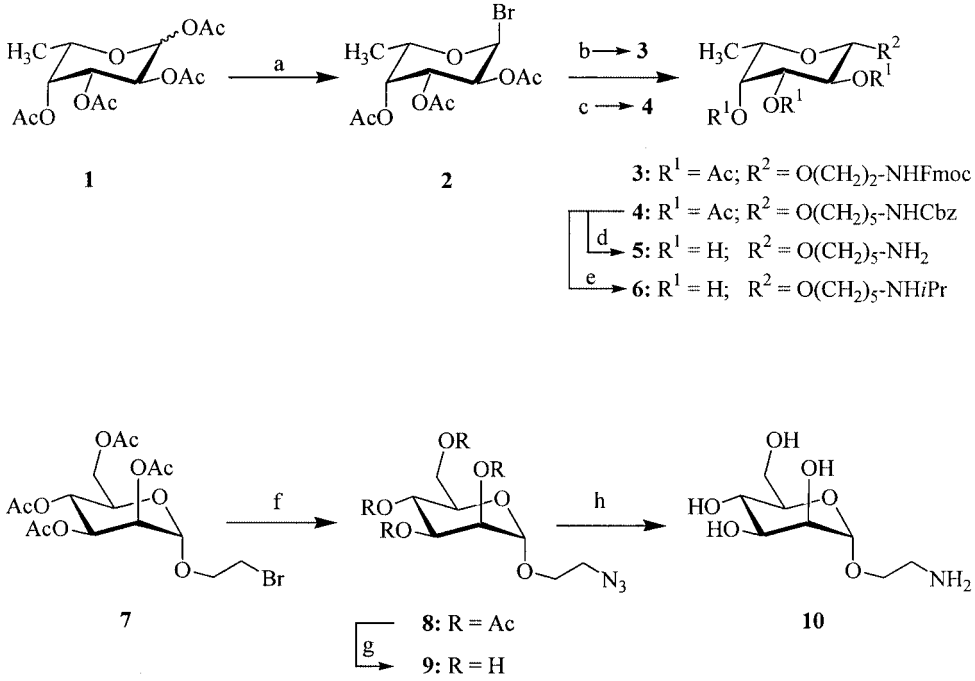
Synthesis of Amino-Functionalized Glycosides

We decided to prepare glycosides carrying the amino function in the aglycon part. Therefore, fucose tetraacetate (**1**) was first converted into Fmoc-protected 2-aminoethyl-β-L-fucoside **3** (Scheme 1) via the fucosyl bromide **2**. However, the low yields of this reaction sequence prompted us to change the amino-protecting group to benzyloxycarbonyl (Cbz). Thus, following a procedure described in the literature,[16] fucosyl bromide **2** was reacted with *N*-Cbz-5-aminopentanol[17] under Helferich conditions to obtain the Cbz-protected 5-aminopentyl-β-L-fucoside **4** in good yield. To prevent *O*→*N* acetyl-group migration, the fully protected compound was first deacetylated under Zemplén conditions[18] and then the Cbz group was removed by hydrogenolysis, making the unprotected aminoalkyl glycoside **5** available. Interestingly, when this deprotection sequence was repeated, the *N*-isopropyl(aminopentyl)fucoside **6** was formed as the only product. This finding can be explained by the presence of acetone in the reaction mixture, leading to **6** as a result of reductive amination. This undesired product **6** eventually became a valuable tool for the elaboration of the synthesis and purification of spacered cluster glycosides (vide infra).

Then, aminoethyl mannoside **10** was easily obtained from the known[19] bromoethyl mannoside **7**, which was transformed into the protected azide **8**, deacetylated to the OH-free mannoside **9**, and finally reduced to the target amine **10** by hydrogenolysis in good yields (Scheme 1).

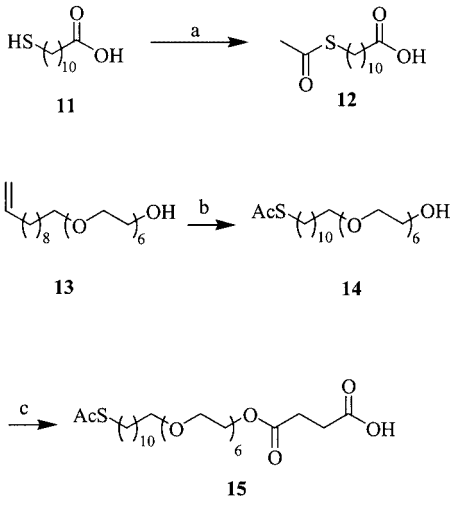
Synthesis of ω-Thio-Functionalized Spacers

The spacers needed for the eventual immobilization of the targeted glycosides on gold have to carry a terminal



Scheme 1. Synthesis of aminoalkyl glycosides **5**, **6** and **10**; reaction conditions: a) HBr/HOAc (33%), DCM, 0 °C to room temp., 3 h, quant.; b) $HO(CH_2)_2NHFmoc$, Ag_2CO_3, DCM, room temp., 4 h, 20%; c) $HO(CH_2)_5NHCbz$, NO_2CH_3/toluene (1:1), $Hg(CN)_2$, room temp., 65%; d) 1. MeOH, NaOMe, room temp., 3 h, 2. MeOH, H_2, Pd/C, 4 h, 70% over 2 steps; e) 1. MeOH, NaOMe, room temp., 3 h, 2. MeOH, acetone, H_2, Pd/C, 3 h, 90%; f) NaN_3, DMF, Bu_4NBr, room temp., 12 h, 89%; g) MeOH, NaOMe, room temp., 2 h; h) MeOH, H_2, Pd/C, 4 h, 90%

thiol or thioacetyl function. In addition, a carboxy function is required for the attachment to the various sugar derivatives by peptide coupling. The commercially available and inexpensive ω-thioundecanoic acid **11** was chosen as a suitable molecule in this regard. *S*-Acetylation to **12** was accomplished in a facile, quantitative reaction using Zn in combination with acetic acid and acetyl chloride (Scheme 2). The resulting thioester could be used in crude form, making tedious purification procedures, as documented in the literature,[20] unnecessary.



Scheme 2. Synthesis of thio-functionalized alkane and hexaethylene glycol spacers; reaction conditions: a) Zn/HOAc, AcCl, DCM, 0 °C to room temp., 1 h, quant.; b) AcSH, THF, room temp., hν, 4 h, 96%; c) succinic anhydride, DIPEA, DCM, reflux 1 h, quant.

For biological investigations with SAMs it is important to avoid unspecific adhesion of biomolecules to the molecular monolayer. Biorepulsive monolayers are obtained when hexaethylene glycol (EG_6) spacers are utilized.[9,21] An oligoethylene moiety resembles a polar unit of the monolayer, covering the gold wafer as a tight film, thus preventing unwanted interactions between the gold surface and proteins used in a bioassay.

The alkene-terminated hexaethylene glycol **13** served as starting material for the synthesis of a thio-functionalized EG-carboxylic acid. It was easily obtained in a substitution reaction involving hexaethylene glycol and 11-bromoundecene.[22] Then, thioacetylation was achieved in a photoreaction with thioacetic acid in THF using AIBN as radical initiator. The reaction mixture was irradiated with a 295 nm UV-filter and a standard Hg-pressure UV-lamp, giving the alcohol **14** in nearly quantitative yields.[23] This procedure also facilitated the critical purification step. The terminal hydroxy group of **14** was then esterified in a reaction with succinic anhydride to form the desired carboxylic acid **15** in 72% overall yield starting from hexaethylene glycol.

Synthesis of ω-Thio-Functionalized Spacered Monosaccharides

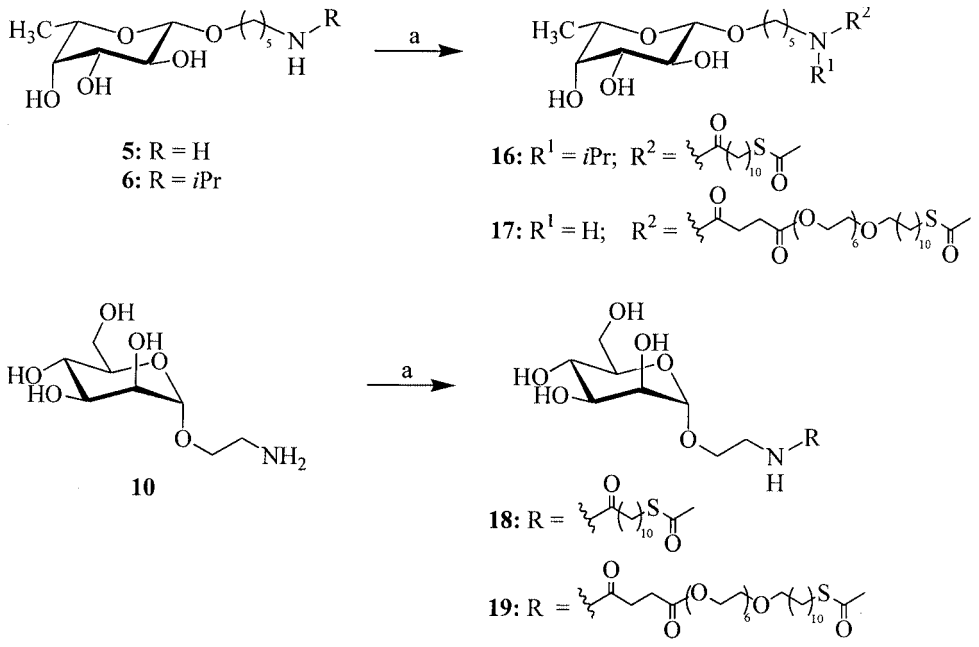
With the desired aminoalkyl glycosides and the ω-thio-functionalized carboxylic acid spacers at hand, peptide coupling was attempted to obtain saccharides suitable for the formation of SAMs. Preliminary coupling experiments with the thio-functionalized undecanoic acid **12** revealed that the products of this reaction are difficult to purify due to their amphiphilic character. However, when the experiments were carried out with the *N*-isopropyl-substituted amine **6**, purification and characterization of the product mixture was easier. Therefore, peptide-coupling conditions were optimized utilizing the secondary amine **6**. When the water-soluble carbodiimide EDC was treated with HOBt in DMF, peptide **16** was obtained in only moderate yields of around 40% (Scheme 3). After the evaluation of the guanidinium-type coupling reagents HBTU and HATU, the mixed anhydride method[16] using isobutyl chloroformate under basic conditions was finally identified as an optimal procedure for this case, yielding **16** in an improved yield of 60%.

These conditions were eventually applied for the peptide-coupling reaction of the valuable hexaethylene glycol carboxylic acid **15** and the aminopentyl fucoside **5** to yield the target glycoside **17**, which is a suitable building block for SAM formation, in 54% yield. In analogy, the spacered mannosides **18** and **19** could be obtained in 65% and an excellent 94% yield, respectively.

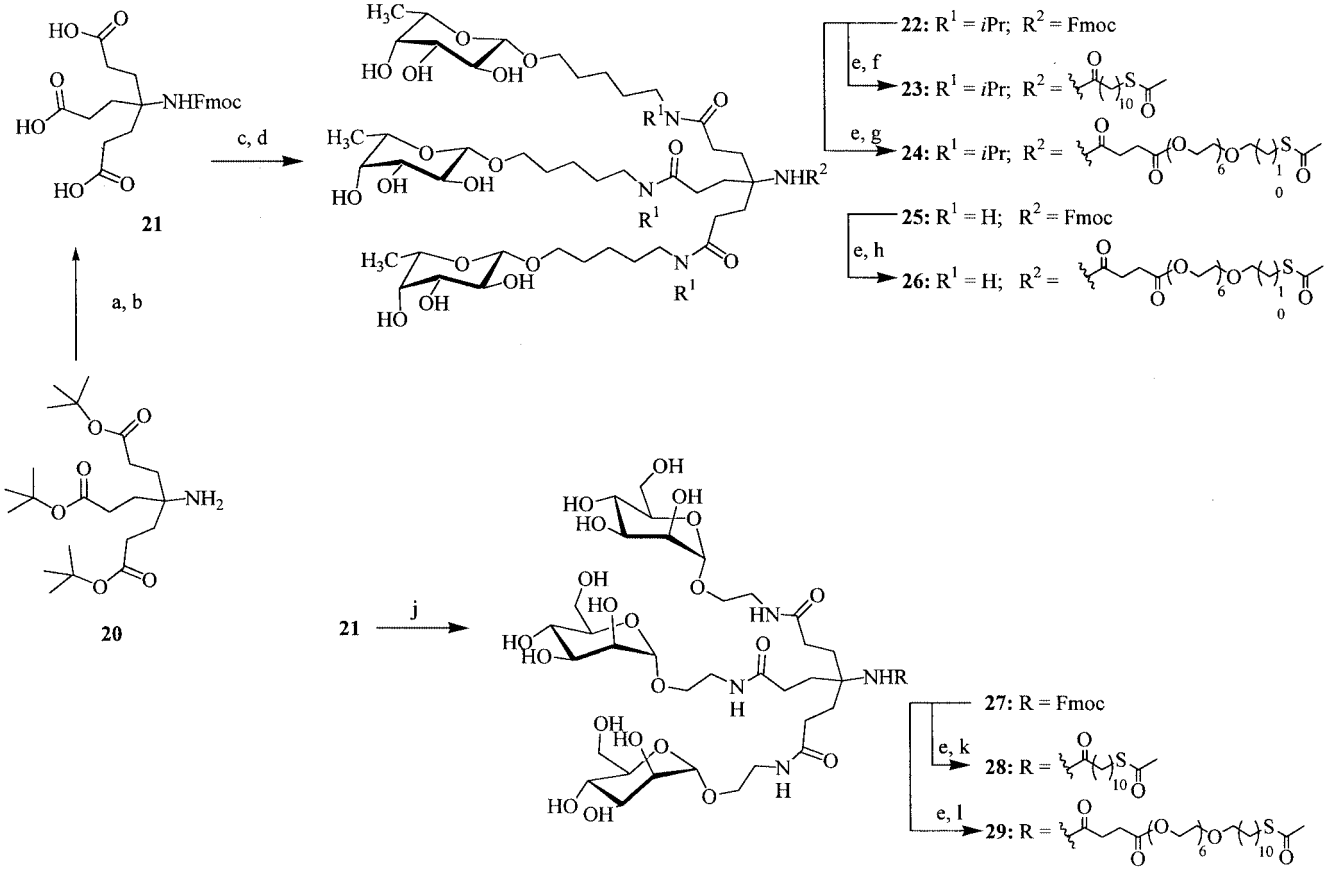
Synthesis of Spacered Cluster Glycosides

The first part of this work led to the four thio-functionalized molecules **16**−**19**, each carrying a monosaccharide moiety, for the formation of glyco-SAMs. Our next goal was to use cluster glycosides along the same synthetic route. The well-established trifunctional dendron **20**[24] was chosen as scaffold molecule for the synthesis of cluster glycosides by peptide coupling. This ester was easily obtained, even on a multigram scale, from nitromethane and acrylic acid *tert*-butyl ester after reduction of the nitro group with Raney-Ni in ethanol.[25] The aminotriester **20** was then converted into the *N*-Fmoc-protected triacid **21** in a one-pot reaction in dichloroethane (Scheme 4). Dichloroethane is the solvent of choice for this reaction because its boiling point is high enough that TFA is not concentrated upon evaporation. Thus, the *N*-protected triacid **21** was obtained in 62% yield over two steps after reversed-phase chromatography (RP-18).

Various coupling conditions were elaborated for the peptide-coupling reaction of aminoalkyl glycosides to the *N*-protected triacid **21**. When carbodiimide-mediated methods were applied, products with structural defects were predominantly observed. Finally, HATU turned out to be a powerful coupling reagent for this case, leading to the fucoside clusters **22** and **25** in the presence of a threefold excess of the fucosides **5** and **6**, respectively (Scheme 4). Steric hindrance due to the *N*-isopropyl group of **6** was no problem in this reaction. Purification of these cluster fucosides was difficult due to the extreme acid lability of the fucosidic bond. However, gel-permeation chromatography with a Sephadex medium and neat methanol as eluent was successfully used for purification. Once again, it turned out that purification of the cluster fucoside **22**, derived from the sec-



Scheme 3. Synthesis of ω-thio-functionalized spacered glycosides; reaction conditions: a) acid **12** or **15**, isobutyl chloroformate, NPr_3, DMF, 0 °C to room temp., 3−6 h, **16**: 60%; **17**: 54%; **18**: 65% and **19**: 94%).



Scheme 4. Synthesis of ω-thio-functionalized spacered cluster glycosides; reaction conditions: a) FmocCl, $C_2H_4Cl_2$, room temp., 3 h; b) TFA, $C_2H_4Cl_2$, room temp., 1 h, 62% over 2 steps; c) **6**, HATU, DIPEA, DMF, room temp., 3 h, 66%; d) **5**, HATU, DIPEA, DMF, room temp., 3 h, 39%; e) morpholine, DMF, room temp., 2 h, quant.; f) **12**, HATU, DIPEA, DMF, room temp., 3 h, 62%; g) **15**, isobutyl chloroformate, NPr_3, DMF, 0 °C to 45 °C, 4 h, 63%; h) **15**, HATU, DIPEA, DMF, room temp., 3 h, 5%; j) **10**, HATU, DIPEA, DMF, room temp., 3 h, 54%; *k*) **12**, isobutyl chloroformate, NPr_3, DMF, 0 °C to room temp., 18 h, 32%; l) **15**, isobutyl chloroformate, NPr_3, DMF, 0 °C to room temp., 2 h, 32%.

ondary *N*-isopropylamine **6**, was easier than purification of **25**. This observation might be explained by the higher molecular weight of **22** and its modulated polarity compared to **25**.

HATU-mediated peptide coupling using mannoside **10** also led to the analogous mannoside cluster **27** in comparable 54% yield. Purification of the trivalent mannoside cluster **27** was possible applying preparative reversed-phase HPLC using an acetonitrile/water gradient containing 0.1% TFA. The same procedure as above caused degradation of the fucosyl clusters.

Next, the trivalent cluster glycosides **22**, **25**, and **27** were coupled to the linear thioacetates **12** and **15**, respectively, at the focal amino group. First, Fmoc-deprotection was carried out in a quantitative reaction with 20% morpholine in DMF. Then, HATU was successfully applied in the peptide-coupling reaction with the acid **12**, leading to the alkyl-spacered target thioacetate **23** in 62% yield. The anhydride method delivered the EG-spacered thioacetate **24** in 63% yield after peptide coupling of the amine derived from **22** with the acid **15**. It turned out that these spacered cluster glycosides possess tensidic properties, with all the associated difficulties regarding their purification. Furthermore, NMR analysis is tricky as in the case of the amide-coupled cluster glycosides **22** and **23**, which occur as two major amide stereoisomers[26] as reflected by two signal sets in the NMR spectrum. High temperature NMR experiments in methanol up to 330 K did not lead to simplification of the spectra. Unfortunately, the EG-spacered fucoside cluster **26** could be obtained in pure form in only 5% yield. This low yield is mainly due to the difficult purification procedure of the acid-labile compound, involving a GPC-HPLC sequence. Only partial purification was achieved by gel chromatography on Sephadex, and subsequent HPLC separation on RP-18 material led to the pure target molecule in reduced yield. On the other hand, the thio-spacered target mannosides **28** and **29** were prepared in 32% and 19% yield, respectively, according to the anhydride route. This finding again shows that the less-sensitive mannosides can be much more conveniently handled than the analogous fucosides.

Conclusion

In conclusion, we have reported the synthesis of spacered monosaccharides and trivalent cluster glycosides of the D-mannose and L-fucose series. Our ongoing work utilizing these molecules has shown that they indeed form SAMs, with varying characteristics, which are currently under investigation, as is a biological evaluation of this system.

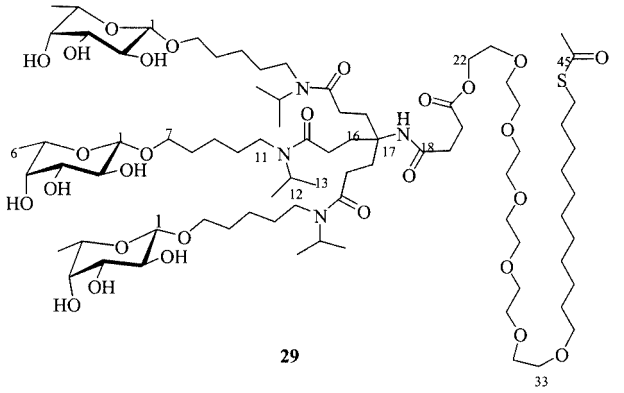
It is important to note that the purification of the target molecules such as **17** and **19**, **26** and **29** is hampered by their amphiphilic character, a fact which often leads to disappointing isolated yields. The β-fucosidic bond proved to be a problem due to its sensitivity towards lowered pH. However, the applied synthetic concept was shown to be a valuable and highly flexible one. We are currently extending our studies on the basis of the results that have been reported here.

Experimental Section

Abbreviations: See ref.[27]

General Remarks: Reactions were carried out in dried glassware under argon or nitrogen and using distilled solvents, unless otherwise noted. THF was dried by distillation from sodium/potassium ketyl, methanol by distillation from magnesium turnings, and dichloromethane by distillation from calcium hydride, all under argon. Commercially available starting materials, reagents and pure DMF were used without further purification. TLC was performed on GF254 silica gel plates (Merck), detection was effected by the use of UV light (254 nm and 366 nm) and with mixtures of either 10% sulfuric acid in ethanol or cerium(IV) sulfate and phosphormolybdate in 10% sulfuric acid followed by heat treatment. Flash chromatography was performed on silica gel 60 (230−400 mesh, particle size 0.040−0.063 mm, Merck). NMR spectra were recorded on Bruker DRX 500 (500 MHz for ^{1}H, 125.47 MHz for ^{13}C) and ARX 300 instruments (300 MHz for ^{1}H, 75.47 MHz for ^{13}C). Spectra were calibrated with respect to the solvent peak ($CDCl_3$: δ = 7.24 ppm for ^{1}H and δ = 77.0 ppm for ^{13}C; [D_4]methanol: δ = 3.35 ppm for ^{1}H and δ = 49.30 ppm for ^{13}C). Assignment of the peaks was achieved with the aid of 2D NMR techniques (^{1}H,^{1}H-COSY and ^{1}H,^{13}C-HSQC). Peak values that could not be unequivocally assigned to one atom, and may therefore be interchangeable, are marked with an asterisk. Hydrogen and carbon atoms within the scaffold are indexed as follows: The sugar residue is numbered as usual from 1 to 6 with the anomeric position being number 1, the atoms of the anomeric spacer moiety then receive higher numbers by consequent numbering starting from the glycoside bond. This is depicted for compound **29** in Figure 2. In the sugar-free spacer molecules the thioacetate is defined as the terminus of the molecule, as exemplified for **15** (Figure 2).
MALDI-TOF mass spectra were recorded on a Bruker Biflex III 19 kV instrument, norharmane (9*H*-pyrido[3,4-*b*]indole) in THF was used as matrix. For sample preparation, a drop of matrix solution was first placed on the target and left to evaporate. Afterwards, a solution of the sample in THF or in methanol was placed on the pre-crystallized matrix.
Even though NMR studies showed no contamination within the samples, correct elemental analyses could not be obtained for most of the reported substances. Even samples that contained no impurities according to analytical HPLC failed to give correct values. Therefore, ESI-MS measurements were also conduced to prove purity and record high-resolution mass spectra. These were performed on a Mariner (Part-No. V800600) instrument. For analytical HPLC chromatography a Merck−Hitachi machine with a diode array detector L-7455 was used with either LiChrosorb® RP-8 7 μm silica or a Chromolith® performance RP-18 100-4.6 mm column. Preparative HPLC chromatography was carried out on a Shimadzu system with Merck columns Hibar® RT250-25 mm with LiChrosorb® RP-8 7 μm silica.

General Procedure A. Peptide Coupling using the Mixed Anhydride Method: The carboxylic acid (1 equiv.) was dissolved 1−3 mL of dry DMF under a nitrogen atmosphere and cooled to 0 °C. Isobutyl chloroformate (1.2 equiv.) and tripropylamine (2.0 equiv.) were added with a syringe and the reaction mixture was stirred for 30 min prior to the addition of a concentrated solution of the amine (1.2 equiv.) in DMF and final addition of tripropylamine (1.0



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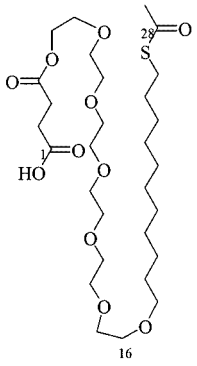

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Figure 2. Numbering of hydrogen and carbon atoms for assignment of NMR spectroscopic data

equiv.) at 0 °C. The reaction mixture was then allowed to warm to room temp. and stirred for 2−6 h.

5-Aminopentyl-*N*-isopropyl-β-L-fucopyranoside (6): Fucoside **4** (143 mg, 0.57 mmol) was dissolved in 10 mL of methanol, a catalytic amount of NaOMe was added and the reaction mixture was stirred for 3 h at room temp. Amberlite IR 120 was then added for neutralization, filtered off, washed several times with methanol and then the solvent was evaporated. The oily crude product was dissolved in 10 mL of methanol containing acetone, Pd/charcoal was added and the reaction mixture was subsequently stirred for 3 h at room temp. The suspension was filtered through a thin bed of celite and the solvent was removed in vacuo to yield **6** as a white foam. Yield: 152 mg (0.52 mmol, M = 291.38) 91%. ^{1}H NMR (500 MHz, $CDCl_3$, TMS): δ = 4.21 (m, 1 H, H-1), 3.89 (dt, J = 6.60, J = 9.54 Hz, 1 H, H-7), 3.67−3.63 (m, 2 H, H-3, H-5), 3.57 (dt, J = 6.61, J = 9.53 Hz, 1 H, H-7′), 3.50−3.49 (m, 2 H, H-2, H-4), 2.88 (sep, J = 6.23 Hz, 1 H, H-12), 2.64 (m, 2 H, 2 H-11), 1.69 (q, J = 6.79 Hz, 2 H, 2 H-8), 1.61−1.55 (m, 2 H, 2 H-10), 1.50−1.44 (m, 2 H, 2 H-9), 1.30 (d, J = 6.60 Hz, 3 H, 3 H-6), 1.13 (d, J = 6.42 Hz, 6 H, 6 H-13) ppm. ^{13}C NMR (125 MHz, $CDCl_3$, TMS): δ = 104.81 (C-1), 75.17 (C-2), 73.01 (C-4), 72.31 (C-3), 71.82 (C-5), 70.51 (C-7), 49.89 (C-12), 47.89 (C-11), 30.58 (C-10), 30.11 (C-8), 24.90 (C-9), 22.18 (2 C-13), 16.77 (C-6) ppm. CI-MS: m/z (%) = 292 (100) [M + H]$^+$, 248 (1.50), 174 (4.46), 146 (24.72) (M = 291.20 calculated for $C_{14}H_{29}NO_5$). ESI-MS: m/z = 292.18 [M + H$^+$] (M = 292.21 calculated for $C_{14}H_{29}NO_5$ + H); m/z = 314.16 [M + Na$^+$] (M = 314.19 calculated for $C_{14}H_{29}NO_5$ + Na).

Succinic Acid Mono[ω-(11-thioacetylundecyl)hexaethylene glycol] Ester (15): DIPEA (20.5 μL, 15.18 mg, 0.117 mmol) was added to a stirred solution of alcohol **15** (100 mg, 0.196 mmol) and succinic anhydride (25.5 mg, 0.255 mmol) in 1.3 mL of dry dichloromethane under nitrogen. The reaction mixture was refluxed for 4 h and subsequently the solvent was removed in vacuo. The obtained reddish oil was dissolved in diethyl ether, acidified with 5 mL of aqueous 1 M HCl and extracted three times with diethyl ether, dried over sodium sulfate and used without further purification. Yield 115 mg (0.188 mmol, M = 610.799) 97%. ^{1}H NMR (500 MHz, $CDCl_3$, TMS): δ = 4.27 (m, 2 H, 2 H-5). 3.66 [m$_c$, 20 H, 2 (H-7, H-8, H-9, H-10, H-11, H-12, H-13, H-14, H-15, H-16)], 3.58−3.57 (m, 2 H, 2 H-6), 3.45 (t, J = 6.85 Hz, 2 H, 2 H-17), 2.86 (t, J = 7.30 Hz, 2 H, 2 H-27), 2.65 [m, 4 H, 2 (H-2, H-3)], 2.32 (s, 3 H, 3 H-29), 1.56 [m$_c$, 4 H, 2 (H-18, H-26)], 1.26 [br. m, 14 H, 2 (H-19, H-20, H-21, H-22, H-23, H-24, H-25)] ppm. ^{13}C NMR (125 MHz, $CDCl_3$, TMS): δ = 196.21 (C-28), 175.00 (C-1), 172.05 (C-4), 71.55 (C-17), 70.67−70.00 (C-7, C-8, C-9, C-10, C-11, C-12, C-13, C-14, C-15, C-16), 68.94 (C-6), 63.85 (C-5), 33.75 (C-2), 30.59 (C-29), 29.64 (C-3), 29.56−28.80 (C-18, C-20, C-21, C-22, C-23, C-24, C-25, C-26, C-27), 26.05 (C-19) ppm. IR (KBr): $\tilde{\nu}$ = 2929 s, 2854 s, 1784 m, 1736 s, 1691 s, 1454 m, 1350 m, 1249 m, 1134 s, 1100 s, 1046 m, 953 m, 907 w, 860, w, 731 w, 627m. CI-MS: m/z (%) = 611 (1.16) [M + H]$^+$, 551 (1.33), 511 (10.88), 435 (1.33), 365 (1.22), 187 (3.81). ESI-MS: m/z = 633.29 [M + Na$^+$] (M = 633.32 calculated for $C_{29}H_{54}O_{11}S$ + Na): m/z = 649.26 [M + K$^+$] (M = 649.30 calculated for $C_{29}H_{54}O_{11}S$ + K).

FucC_5N*i*PrC_{11}SAc (16):[27] The reaction was carried out according to the general procedure A using carboxylic acid **12** (51.8 mg, 0.200 mmol) in 1.5 mL of DMF, isobutyl chloroformate (32.8 mg, 0.240 mmol) and tripropylamine (76.1 μL 0.400 mmol). After 20 min a solution of fucoside **5** (59.8 mg, 0.240 mmol) and tripropylamine (38.01 μL, 0.200 mmol) in 0.5 mL of DMF was syringed in dropwise. After 6 h the solvent was evaporated and the pale-yellow crude oil was purified by flash chromatography (MeOH/DCM, 1:20 → 1:10). Yield 59 mg (0.120 mmol, M = 491.68) 60%. ^{1}H NMR (500 MHz, $CDCl_3$, TMS): δ = 4.21 (m$_c$, 1 H, H-1), 4.04 (m, 1 H, H-12), 3.90 (m$_c$, 1 H, H-7), 3.71 (br., 1 H, H-4), 3.64−3.53 (m, 3 H, H-2, H-3, H-5), 3.51 (m$_c$, 1 H, H-7′), 3.12 (m$_c$, 2 H, 2 H-11), 2.86 (t, J = 7.33 Hz, 2 H, 2 H-25), 2.32 (m, 4 H, 3 H-27, H-15), 2.26 (m$_c$, 1 H, H-15′), 1.64 (m$_c$, 2 H, 2 H-8), 1.60−1.52 [m$_c$, 6 H, 2 (H-10, H-16, H-24)], 1.375 (m, 2 H, 2 H-9), 1.33 (d, J = 6.41 Hz, 3 H-6), 1.27 [m$_c$, 14 H, 2 (H-17, H-18, H-19, H-20, H-21, H-22, H-23)], 1.18 (d, J = 6.60 Hz, 3 H, 3 H-13), 1.12 (d, J = 6.62 Hz, 3 H, 3 H-13) ppm. ^{13}C NMR (125 MHz, $CDCl_3$, TMS): δ = 196.02 (C-26), 172.91 (C-14), 172.56 (C-14′), 103.01 (C-1), 74.00 (C-3), 71.49 (C-4), 71.39 (C-2), 70.45 (C-5), 69.64 (C-7), 48.25 (C-12), 40.92 (C-11), 33.82 (C-15), 31.27 (C-16), 30.58 (C-26), 29.44 (C-10), 29.40−29.10 (C-17, C-18, C-19, C-20, C-21, C-22, C-23), 28.91 (C-8), 28.73, 25.52, 23.92, 21.32 (C-13, C-13′), 16.32 (C-6) ppm. MALDI-TOF: m/z = 556 [M + Na]$^+$ and m/z = 572 [M + K]$^+$ (M = 533.34 calculated for $C_{27}H_{51}NO_7S$).

FucC_5NHsucEG$_6$C$_{11}$SAc (17): The reaction was carried out according to the general procedure A using carboxylic acid **15** (370 mg, 0.605 mmol) in 3 mL of DMF, isobutyl chloroformate (94.3 mg, 0.727 mmol) and tripropylamine (230.5 μL, 1.21 mmol). After 15 min a solution of fucoside **5** (181.2 mg, 0.727 mmol) and tripropylamine (115 μL, 0.606 mmol) in 0.5 mL of DMF was syringed in dropwise. After 6 h the solvent was evaporated and the yellow, highly viscous crude oil was purified by flash chromatography (MeOH/DCM, 1:20 → 1:10). Yield 280 mg (0.333 mmol, M = 841.49) 55%. ^{1}H NMR (500 MHz, $CDCl_3$, TMS): δ = 4.23 (m, 2

H, 2 H-16), 4.18 (m_c, 1 H, H-1), 3.86 (dt, J = 6.05, J = 9.54 Hz, 1 H, H-7), 3.71 (m_c, 2 H, 2 H-17), 3.65 [m_c, 20 H, H-3, H-5, 2 (H-18, H-19, H-20, H-21, H-22, H-23, H-24, H-25, H-26)], 3.60−3.57 (m, 4 H, H-2, H-4, 2 H-27), 3.53 (dt, J = 6.40, J = 9.54 Hz, 1 H, H-7′), 3.44 (m, 2 H, 2 H-28), 3.18 (m, 2 H, 2 H-11), 2.89 (m_c, 2 H, 2 H-38), 2.64 (t, J = 6.97 Hz, 2 H, 2 H-13), 2.49 (t, J = 6.96 Hz, 2 H, 2 H-14), 2.32 (s, 3 H, 3 H-40), 1.65 (m_c, 2 H, 2 H-8), 1.59−1.50 [m, 6 H, 2 (H-10, H-29, H-37)], 1.46−1.42 (m, 2 H, 2H-9), 1.35−1.31 [m_c, 14 H, 2 (H-30, H-31, H-32, H-33, H-34, H-35, H-36)], 1.27 (d, J = 6.6 Hz, 3 H, 3 H-6) ppm. ^{13}C NMR (125 MHz, $CDCl_3$, TMS): δ = 197.61 (C-39), 174.24 (C-12), 174.11 (C-15), 104.82 (C-1), 75.19 (C-2), 73.05 (C-3), 72.37 (C-4), 72.35 (C-28), 71.86 (C-5), 71.57 (C-18, C-19, C-20, C-21, C-22, C-23, C-24, C-25, C-26), 71.17 (C-27), 70.49 (C-7), 70.08 (C-17), 64.85 (C-16), 40.37 (C-11), 31.49 (C-14), 30.75 (C-40), 30.56−30.40 (C-13, C-30, C-31, C-32, C-33, C-34, C-35, C-36, C-37, C-38), 30.12 (C-29), 29.78 (C-10), 27.23 (C-8), 24.42 (C-9), 16.78 (C-6) ppm. MALDI-TOF: m/z = 864 [M + Na]$^+$ (M = 841.49 calculated for $C_{40}H_{75}NO_{15}S$). ESI-MS: m/z = 664.42 [M + Na$^+$] (M = 664.47 calculated for $C_{40}H_{75}NO_{15}S$ + Na).

ManC_2NHC_{11}SAc (18): The reaction was carried out according to the general procedure A using carboxylic acid **15** (77 mg, 0.296 mmol) in 1.5 mL of DMF, isobutyl chloroformate (46 μL, 0.355 mmol) and tripropylamine (112 μL, 0.6 mmol). After 30 min amine **10** (79.2 mg, 0.355 mmol) in 0.5 mL of DMF and tripropylamine (56 μL, 0.3 mmol) were added to the clear reaction mixture which was then warmed to room temp. over 2 h and stirred overnight. The solvent was removed in vacuo and the residue was taken up in 15% acetonitrile (aq.) and purified by HPLC chromatography water/acetonitrile, 30% → 70% acetonitrile, 60 min, 10 mL/min, t_R = 35 min) to yield a white foam. Yield: 89.4 mg (192 mmol, M = 465.60) 65%. ^{1}H NMR (500 MHz, $CDCl_3$, TMS): δ= 4.75 (br. d, 1 H, H-1), 3.82 (dd, J = 2.2, J = 9.53 Hz, 1 H, H-6), 3.80 (dd, J = 1.65, J = 3.48 Hz, 1 H, H-2), 3.74 (m_c, 1 H, H-7), 3.67 (m_c, 2 H, H-3, H-6′), 3.59 (m, "t", J = 9.54 Hz, 1 H, H-4), 3.52 (m_c, 2 H, H-5, H-7′), 3.41−3.34 (m, 2 H, 2 H-8), 2.85 (t, J = 7.16 Hz, 2 H, 2 H-19), 2.30 (s, 3 H, 3 H-21), 2.19 (t, J = 2.52 Hz, 2 H, 2 H-10), 1.59 (m_c, 2 H, 2 H-18), 1.53 (m_c, 2 H, 2 H-11), 1.30 [br., 12 H, 2 (H-12, H-13, H-14, H-15, H-16, H-17)] ppm. ^{13}C NMR (125 MHz, $CDCl_3$, TMS): δ = 197.67 (C-20), 176.58 (C-9), 101.72 (C-1), 74.80 (C-5), 72.56 (C-3), 72.11 (C-2), 68.80 (C-4), 67.30 (C-7), 62.93 (C-6), 40.27 (C-8), 37.10 (C-10), 30.80 (C-21), 30.54−29.83 (C-11, C-12, C-13, C-14, C-15, C-16, C-17, C-18), 27.07 (C-19) ppm. MALDI-TOF: m/z = 488 [M + Na]$^+$ (M = 465.24 calculated for $C_{21}H_{39}NO_8S$). ESI-MS: m/z = 488.19 [M + Na$^+$] (M = 488.22 calculated for $C_{21}H_{39}NO_8S$ + Na).

ManC_2NHsucEG$_6$C$_{11}$SAc (19): The reaction was carried out according to the general procedure A using carboxylic acid **15** (226 mg, 0.370 mmol) in 1.5 mL of DMF, isobutyl chloroformate (57.6 μL, 0.444 mmol) and tripropylamine (140 μL, 0.740 mmol). After 30 min a solution of mannoside **10** (99.1 mg, 0.444 mmol) and tripropylamine (70 μL, 0.370 mmol) in 1 mL of DMF was syringed in dropwise. After 3 h the solvent was evaporated and the yellow, highly viscous crude oil was purified by flash chromatography (MeOH/DCM, 1:20 → 1:10). Yield 304 mg (0.373 mmol, M = 815.43) 94%. ^{1}H NMR (500 MHz, $CDCl_3$, TMS): δ = 4.77 (br., 1 H, H-1), 4.22 (m, 2 H, 2 H-13), 3.8 (dd, J = 2.40, J = 11.74 Hz, 1 H, H-6), 3.81 (m_c, 1 H, H-2), 3.75 (m, 1 H, H-7), 3.71−3.69 (m, 4 H, H-3, H-6′, 2 H-14), 3.64 [br., 20 H, 2 (H-15, H-16, H-17, H-18, H-19, H-20, H-21, H-22, H-23, H-24)], 3.58 (m_c, 2 H, H-4, H-5), 3.53 (m_c, 1 H, H-7′), 3.45 (m, "t", J = 7.78 Hz, 2 H, 2 H-25), 3.42 (m_c, 1 H, H-8), 3.37 (m_c, 1 H, H-8′), 2.86 (t, J = 7.34 Hz, 2 H, 2 H-35), 2.64 (m, 2 H, 2 H-10), 2.53 (m_c, 2 H, 2 H-11), 2.30 (s, 3 H, 3 H-37), 1.59−1.53 [m, 4 H, 2 (H-26, H-34)], 1.26 [br., 14 H, 2 (H-27, H-28, H-29, H-30, H-31, H-32, H-33)] ppm. ^{13}C NMR (125 MHz, $CDCl_3$, TMS): δ = 197.43 (C-36), 174.40 (C-9), 174.18 (C-12), 101.61 (C-1), 74.68 (C-5), 73.61, 72.49 (C-3), 72.30 (C-25), 71.99 (C-2), 70.51−71.10 (C-15, C-16, C-17, C-18, C-19, C-20, C-21, C-22, C-23, C-24), 68.60 (C-4), 67.20 (C-7), 64.82 (C-13), 62.89 (C-6), 62.17, 40.30 (C-8), 31.29 (C-10), 30.68 (C-11), 30.63−29.81 (C-26, C-27, C-28, C-29, C-30, C-31, C-32, C-33, C-34), 29.73 (C-35), 27.15 ppm. MALDI-TOF: m/z = 838 [M + Na]$^+$ (M = 815.43 calculated for $C_{37}H_{69}NO_{16}S$). ESI-MS: m/z = 838.38 [M + Na$^+$] (M = 838.42 calculated for $C_{37}H_{69}NO_{16}S$ + Na).

4-(2-Carboxyethyl)-4-(9-fluorenylmethoxycarbonylamino)heptane-1,7-diacid (21): Fmoc chloride (1.21 g, 4.64 mmol) was mixed with aminotriester **20** (1.93 g, 4.64 mmol) and dissolved in 40 mL of dichloromethane. DIPEA (1 mL) was then added and the mixture was stirred for 1 h at room temp. The solvent was removed in vacuo, the residue was taken up in 10 mL of DCM and 5 mL of TFA was added. After 3 h of additional stirring the solution was diluted with 50 mL of $C_2H_4Cl_2$ and the solvent was removed in vacuo at room temp. Column chromatography on RP-18 silica gel (MeCN/H_2O, 1:1) and subsequent lyophilization yielded a colourless powder. Yield: 1.36 g (2.89 mmol, M = 469.35) 62%. HPLC: t_R = 24.38 min [250/4 LiChrosorb 7 μm C8, water/acetonitrile, 10% → 80% acetonitrile, 60 min, 1 mL/min]. ^{1}H NMR (300 MHz, MeOD): δ = 7.77 (d, J = 7.08 Hz, 2 H, Ar-H), 7.08 (d, J = 7.19 Hz, 2 H, Ar-H), 7.32 (m_c, 4 H, Ar-H), 4.35 (m, 2 H, 2 H-6), 4.18 (m, 1 H, H-7), 2.24 (m_c, 6 H, 6 H-2), 1.94 (m_c, 6 H, 6 H-3) ppm. ^{13}C NMR (75.47 MHz, MeOD): δ = 177.10 (C-1), 156.52 (C-5), 145.36, 142.60, 128.73, 128.16, 126.18, 120.88 (Ar-C), 66.78 (C-6), 57.42 (C-4), 30.55, 29.11 (C-2, C-3). ESI-MS: m/z = 492.12 [M + Na$^+$] (M = 492.16 calculated for $C_{25}H_{27}NO_8$ + Na).

(FucC_5N*i*Pr)$_3$NHFmoc (22): Fucoside **6** (123 mg, 0.42 mmol) was mixed with triacid **21** (66 mg, 0.14 mmol) and HATU (171 mg, 0.45 mmol). The mixture was dissolved in 2 mL of dry DMF, the solution cooled to 0 °C, (92 μL, 0.54 mmol) of DIPEA added and the reaction mixture stirred for 6 h at room temp. The solvent was removed in vacuo and the residue was taken up in methanol and purified by gel-permeation chromatography on Sephadex LH-20 to yield a white foam. Yield: 120 mg (93 μmol, M = 1289.59) 66%. ^{1}H NMR (500 MHz, MeOD): δ = 7.81 (m, 2 H, Ar-H), 7.69 (m, 2 H, Ar-H), 7.39 (m, 2 H, Ar-H), 7.32 (m, 2 H, Ar-H), 4.52 (m_c, 1 H, H-12), 4.31 (m_c, 2 H, 2 H-19), 4.21 (m_c, 3 H, 2 H-12, H-20), 4.15 (m, 3 H, 3 H-1), 3.79 (m_c, 3 H, 3 H-7), 3.58 (m, 6 H, 3 H-4, 3 H-5), 3.51 (m_c, 3 H, 3 H-7), 3.44 (m_c, 6 H, 3 H-2, 3 H-3), 3.16 (m_c, 6 H, 6 H-11), 2.36 (m, 6 H, 6 H-15), 1.97 (m, 6 H, 6 H-16), 1.63 (m, 6 H, 6 H-8), 1.56 (m, 6 H, 6 H-9), 1.36 (m, 6 H, 6 H-10), 1.25 (m, 9 H, 9 H-6), 1.15 (m, 18 H, 18 H-13).^{13}C NMR (125.47 MHz, MeOD): δ = 174.79, 174.21 (C-14), 156.96 (C-18), 145.33, 142.64, 128.88, 128.26, 126.26, 121.06 (Ar-C), 104.84, 104.81 (C-1), 75.19 (C-2), 73.06, 7302 (C-4), 72.33, (C-3), 71.84 (C-5), 70.55, 70.35 (C-7), 67.32 (C-20), 58.11 (C-17), 50.01, 49.85 (C-12), 47.83, 45.04, 42.32 (C-11), 32.14, 31.48, 30.51 (C-15, C-16), 30.39, 30.29, 24.84, 24.62 (CH_2), 21.53, 20.65 16.82, 16.79 (CH_3) ppm. MALDI-TOF: m/z = 1313 [M + Na]$^+$ (M = 1288.76 calculated for $C_{67}H_{108}N_4O_{20}$). ESI-MS: m/z = 1311.68 [M + Na$^+$] (M = 1311.74 calculated for $C_{67}H_{108}N_4O_{20}$ + Na).

(FucC_5N*i*Pr)$_3$C$_{11}$SAc (23): Fmoc-protected fucoside **22** (120 mg, 93 μmol) was dissolved in 4 mL of dry DMF, 1 mL of morpholine was added and the solution was stirred for 1 h at room temp. The solvent was removed in vacuo, the residue was mixed with 11-thioacetylundecanoic acid **12** (30 mg, 0.12 mmol) and HATU

(46 mg, 0.12 mmol) and subsequently the mixture was dried for 1 h in vacuo. Under an argon atmosphere the compounds were dissolved in 5 mL of dry DMF, DIPEA (25 μL, 0.15 mmol) was added and the solution was stirred for 18 h at room temp. The solvent was removed in vacuo, the residue was taken up in methanol and purified by gel-permeation chromatography on Sephadex LH-20 to yield a colourless foam. Yield: 76 mg (58 μmol, M = 1309.73) 62%. HPLC: t_R = 24.75 min [250/4 LiChrosorb 7μm C8, water/acetonitrile, 20% → 90% acetonitrile, 60 min, 1 mL/min]. The NMR spectroscopic data showed clearly the presence of two amide rotamers in a 1:0.9 ratio, which can undoubtedly be assigned in accordance to the doubled set of signals for the isopropyl group and the methylene signals close to it. Here, the chemical shifts of both isomers are listed. ^{1}H NMR (500 MHz, 300 K, $CDCl_3$): δ = 4.53 (br. sept, 3 H, 3 H-12), 4.19 (m_c, 3 H, 3 H-1), 4.06 (br. sept, 2 H, 3 H-12), 3.91 (m_c, 3 H, 3 H-7), 3.72 (m_c, 3 H, 3 H-4), 3.59 [m_c, 9 H, 3 (H-2, H-3, H-5)], 3.49 (m_c, 3 H, 3 H-7′), 3.13 (br. m, H-11), 2.83 (br. t, 2 H, H-28), 2.35 (m_c, 6 H, CH_2), 2.31 (br. s, 3 H, 3 H-30), 2.15 (m_c, 8 H, CH_2), 1.60 (m_c, 16 H, CH_2, H-27), 1.28 (m_c, 13 H, CH_2, 9 H-6), 1.18 (d, J = 6.42 Hz, 9 H, 9 H-13), 1.12 (d, J = 6.79 Hz, 9 H, 9 H-13) ppm. ^{13}C NMR (125.47 MHz, $CDCl_3$): δ = 196.21 (C-29), 173.61 (C-14), 173.44 (C-14), 172.97 (C-18), 103.34 (C-1), 103.17 (C-1), 74.43, 74.11, 71.77, 71.71, 71.45, 70.62 (C-2, C-3, C-4, C-5), 69.03 (C-7), 57.62 (C-17), 48.66, 46.18 (C-12), 43.88, 41.15, 37.72, 31.10, 30.78 (C-30), 29.63−28.95, 26.18, 26.03, 24.16, 23.52 (CH_2), 21.50, 20.65 (C-13), 16.56 (C-6) ppm. MALDI-TOF: m/z = 1332 [M + Na]$^+$ (M = 1308.82 calculated for $C_{65}H_{120}N_4O_{20}S$).

(FucC$_5$N*i*Pr)$_3$sucEG$_6$C$_{11}$SAc (24): The protected cluster **22** (60 mg, 0.046 mmol) was dissolved in 1.5 mL of DMF at room temp. under nitrogen, 0.3 mL of morpholine was added and the reaction mixture was stirred for 2 h. The solvents were removed in vacuo and 50 mg of a colourless solid was obtained and used without further purification. The coupling was performed according to procedure A using carboxylic acid **15** (25.8 mg, 0.042 mmol) in 0.5 mL of DMF, isobutyl chloroformate (6 μL, 0.046 mmol) and tripropylamine (16 μL, 0.082 mmol). After 30 min free amine (50 mg, 0.046 mmol) was dissolved in 1 mL of DMF and added to the solution. Stirring was maintained at room temp. overnight prior to solvent removal in vacuo. The crude yellow oily product was subjected to HPLC purification (t_R = 48.20 min; A = water, B = acetonitrile, 0% B → 70% B, 5 min, 70% B → 30% B, 60 min, 10 mL/min) to give a colourless oil. Yield: 48 mg (0.029 mmol, M = 1659.02) 63%. The NMR spectroscopic data clearly showed the presence of two amide rotamers in a 1:0.8 ratio, which can undoubtedly be assigned in accordance to the doubled set of signals for the isopropyl group and the methylene signals close to it. Here, the chemical shifts of both isomers are listed. ^{1}H NMR (500 MHz, MeOD): δ = 4.54 (m_c, 1 H, H-12), 4.25−4.20 (m, 7 H, 3 H-1, 2 H-12, 2 H-22), 3.89 (m_c, 3 H, 3 H-7), 3.73 (m_c, 3 H, 3 H-23), 3.68 [m_c, 21 H, 3 H-5, 2 (H-24, H-25, H-26, H-27, H-28, H-29, H-30, H-31, H-32)], 3.64 (m_c, 3 H, 3 H-4), 3.62 (m, 2 H, 2 H-33), 3.57 (m_c, 3 H, 3 H-7′), 3.51 (m_c, 8 H, 3 H-2, 3 H-3, 2 H-34), 3.28 (m, 3 H, 3 H 11), 3.20 (m, 3 H, 3 H-11′), 2.90 (t, J = 7.15 Hz, 2 H, 2 H-44), 2.68 (m_c, 2 H, 2 H-19), 2.53 (m_c, 2 H, 2 H-20), 2.45 (m_c, 3 H, 3 H-15), 2.39 (m_c, 3 H, 3 H-15′), 2.35 (s, 3 H, 3 H-46), 2.05 (m_c, 6 H, 6 H-16), 1.68 (m, 9 H, 6 H-8, 3 H-10), 1.60 (m, 7 H, 3 H-10′, 2 H-35, 2 H-43), 1.49−1.34 [m, 20 H, 6 H-9, 2 (H-36, H-37, H-38, H-39, H-40, H-41, H-42)], 1.30 (d, J = 6.42 Hz, 9 H, 9 H-6), 1.26 (d, J = 6.61 Hz, 10 H, 10 H-13), 1.20 (d, J = 6.78 Hz, 8 H, 8 H-13′) ppm. ^{13}C NMR (125 MHz, MeOH, TMS): δ = 197.56 (C-45), 174.78 (C-14), 174.30 (C-18), 143.60 (C-21), 104.80 (C-1), 75.19 (C-33), 75.16 (C-3), 73.02 (C-4), 72.33 (C-2), 72.31 (C-34), 72.30 (C-5), 71.81−71.13 (C-7, C-23, C-24, C-25, C-26, C-27, C-28, C-29, C-30, C-31, C-32), 64.82 (C-22), 59.01 (C-17), 49.96 (C-12), 47.79 (C-12′), 45.0 (C-11), 42.27 (C-11′), 32.10 (C-20), 32.09 (C-8), 31.90 (C-16), 30.71−30.51 (C-10, C-35, C-36, C-37, C-38, C-39, C-40, C-41, C-42, C-43, C-46), 30.15 (C-19), 29.84 (C-44), 29.78 (C-15), 27.10 (C-9), 21.67 (C-13), 20.67 (C-13′), 16.80 (C-6). MALDI-TOF: m/z = 1683.62 [M + Na]$^+$ (M = 1682.00 calculated for $C_{81}H_{150}N_4O_{28}S$ + Na); m/z = 1698.64 [M + K]$^+$ (M = 1698.12 calculated for $C_{81}H_{150}N_4O_{28}S$ + K). ESI-MS: m/z = 1682.07 [M + Na$^+$] (M = 1682.01 calculated for $C_{81}H_{150}N_4O_{28}S$ + Na).

(FucC$_5$NH)$_3$NHFmoc (25): Fucoside **5** (105 mg, 0.42 mmol) was mixed with triacid **21** (66 mg, 0.14 mmol) and HATU (171 mg, 0.45 mmol). The mixture was dissolved in 2 mL of dry DMF, the solution cooled to 0 °C, DIPEA (92 μL, 0.54 mmol) added and the reaction mixture stirred for 6 h at room temp. The solvent was removed in vacuo, the residue was taken up in methanol and purified by gel-permeation chromatography on Sephadex LH-20 to yield a white foam. Yield: 64 mg (55 μmol, M = 1163.35) 39%. HPLC: t_R = 39.41 min [250/4 LiChrosorb 7 μm C8, water/acetonitrile, 0% → 70% acetonitrile, 60 min, 1 mL/min]. ^{1}H NMR (500 MHz, MeOD): δ = 7.82 (br. d, J = 7.52 Hz, Ar-H), 7.69 (m, 2 H, Ar-H), 7.39 (m, 2 H, Ar-H), 7.31 (m, 2 H, Ar-H), 4.32 (m, 2 H, 2 H-17), 4.20 (m, 1 H, H-18), 4.16 (m, 3 H, 3 H-1), 3.84 (m, 3 H, 3 H-7), 3.58 (m, 6 H, 3 H-4, 3 H-5), 3.51 (m_c, 3 H, 3 H-7′), 3.45 (m, 6 H, 3 H-2, 3 H-3), 3.15 (m, 6 H, 6 H-11), 2.16 (m, 6 H, 6 H-13), 1.92 (m, 6 H, 6 H-14), 1.62 (m, 6 H, 6 H-8), 1.51 (m, 6 H, 6 H-9), 1.41 (m, 6 H, 6 H-10), 1.25 (d, J = 6.4 Hz, 9 H, 9 H-6) ppm. ^{13}C NMR (125.47 MHz, MeOD): δ = 175.69, 175.58 (C-12), 145.44, 142.61, 128.78, 128.19, 126.31, 120.95 (Ar-C), 104.79 (C-1), 75.16 (C-2), 73.04 (C-4), 72.33, (C-3), 71.84 (C-5), 70.64 (C-7), 67.11 (C-17), 57.89 (C-15), 40.42 (C-11), 30.39, 30.08 (C-13, C-14), 30.71 (C-8), (C-9), 24.44 (C-10), 16.81 (C-6) ppm. MALDI-TOF: m/z = 1186 [M + Na]$^+$ (M = 1162.61 calculated for $C_{58}H_{90}N_4O_{20}$). ESI-MS: m/z = 1185.55 [M + Na$^+$] (M = 1185.60 calculated for $C_{58}H_{90}N_4O_{20}$ + Na).

(FucC$_5$NH)$_3$sucEG$_6$C$_{11}$SAc (26): Fmoc-protected fucoside **25** (60 mg, 37 μmol) was dissolved in 4 mL of dry DMF, 1 mL of morpholine was added and the solution was stirred for 1 h at room temp. The solvent was removed in vacuo, the residue was mixed with EG$_6$-alkyl thioacetate **15** (271 mg, 0.44 mmol) and HATU (171 mg, 0.45 mmol), and then the mixture was dried for 1 h in vacuo. Under argon atmosphere the compounds were dissolved in 5 mL of dry DMF, DIPEA (85 μL, 0.5 mmol) was added and the solution was stirred for 48 h at room temp. The solvent was removed in vacuo, the residue was taken up in methanol and purified by gel-permeation chromatography on Sephadex LH-20. Preparative HPLC of the residue on RP-8 silica gel (A = water, B = acetonitrile, 5% B → 80% B, 10 mL/min, 60 min, t_R = 52 min) yielded a colourless foam. Yield: 24 mg (15 μmol, M = 1533.89) 5%. ^{1}H NMR (500 MHz, MeOD): δ = 4.22 (m_c, 2 H, 2 H-20), 4.17 (m_c, 3 H, 3 H-1), 3.85 (m_c, 3 H, 3 H-7), 3.70 (m_c, 2 H, 2 H-21), 3.64 [23 H, 3 H-5, 2 (H-22, H-23, H-24, H-25, H-26, H-27, H-28, H-29, H-30, H-31)], 3.59 (5 H, 3 H-4, 2 H-32), 3.53 (m_c, 3 H, 3 H-7′), 3.46 (m_c, 6 H, 3 H-2, 3 H-3), 3.17 (m_c, 6 H, 6 H-11), 2.86 (t, J = 7.20 Hz, 2 H, 2 H-42), 2.65 (m_c, 2 H, 2 H-17), 2.50 (m_c, 2 H, 2 H-18), 2.30 (s, 3 H, 3 H-44), 2.18 (m_c, 6 H, 6 H-13), 1.96 (m_c, 6 H, 6 H-14), 1.64 (quint, J = 6.97 Hz, 6 H, 6 H-8), 1.54 (m_c, 10 H, 6 H-10, 3 H-33, 3 H-41), 1.41 (m_c, 6 H-9), 1.31 (m_c, H-34, H-35, H-36, H-37, H-38, H-39, H-40), 1.26 (d, J = 6.45 Hz, 12 H, 12 H-6) ppm. ^{13}C NMR (125.47 MHz, MeOH): δ = 175.77 (C-19), 174.89 (C-16), 104.83 (C-1), 75.21 (C-2), 73.06 (C-4), 72.37 (C-3), 71.86 (C-

5), 71.58, 71.17, 70.46 (C-7), 70.08 (C-18), 64.99 (C-17), 59.04 (C-15), 40.42 (C-11), 31.75, 31.31, 30.74 (C-13, C-14), 30.74, 30.69, 30.57, 30.42, 30.19, 30.13, 29.86, 29.79 (C-30), 24.77 (C-42), 16.81 (C-6) ppm. MALDI-TOF-MS: *m/z* = 1557 $[M + Na]^+$ (*M* = 1532.87 calculated for $C_{72}H_{132}N_4O_{28}S$). ESI-MS: *m/z* = 789.38 $[M + 2\ Na^+]$ (*M* = 789.42 calculated for $C_{72}H_{132}N_4O_{28}S$ +2 Na); *m/z* = 1555.82 $[M + Na^+]$ (*M* = 1555.86 calculated for $C_{72}H_{132}N_4O_{28}S$ + Na).

$(ManC_2)_3NHFmoc$ (27): Mannoside **10** (100 mg, 0.45 mmol) was mixed with triacid **21** (70 mg, 0.15 mmol) and HATU (171 mg, 0.45 mmol). The mixture was dissolved in 10 mL of dry DMF, the solution cooled to 0 °C, 100 μL of DIPEA added and the reaction mixture tirred for 4 h at room temp. The solvent was removed in vacuo, the residue was taken up in 15% acetonitrile (aq.) and purified by HPLC chromatography (A = water, B = acetonitrile, 20% B → 50% B, 70 min, 10 mL/min, t_R = 32 min) to yield a white foam. Yield: 90 mg (82 μmol, *M* = 1085.11) 54%. HPLC: t_R = 18.91 min [250/4 LiChrosorb 7 μm C8, A = water, B = acetonitrile, 0% B → 0% B 10 min, 0% B → 75% B, 50 min, 1 mL/min]. ^1H NMR (500 MHz, MeOD): δ = 7.79 (m, 2 H, Ar-H), 7.68 (m, 2 H, Ar-H), 7.37 (m, 2 H, Ar-H), 7.31 (m, 2 H, Ar-H), 4.76 (d, *J* = 1.4 Hz, 3 H, 3 H-1), 4.34 (br. d, 2 H, 2 H-14), 4.19 (br. t, 1 H, H-15), 3.84 (dd, *J* = 2.2, *J* = 11.7 Hz, 3 H, 3 H-6), 3.84 (dd, *J* = 1.8, *J* = 3.5 Hz, 3 H, 3 H-6), 3.76 (m, 3 H, 3 H-7), 3.69 (m_c, 6 H, 3 H-3, 3 H-6′), 3.58 (m, "t", 3 H, 3 H-4), 3.55 (m_c, 6 H, 3 H-5, 3 H-7′), 3.37 (m_c, 6 H, 6 H-8), 2.15 (m_c, 6 H, 6 H-10), 1.95 (m_c, 6 H, 6 H-11) ppm. ^{13}C NMR (125.47 MHz, MeOD): δ = 175.99 (C-9), 156.66 (C-13), 145.43, 142.61, 128.76, 128.19, 126.31, 120.92 (Ar-C), 101.64 (C-1), 74.83 (C-5), 72.51 (C-3), 72.05 (C-2), 68.67 (C-4), 67.22 (C-7), 67.08 (C-14), 62.98 (C-6), 57.88 (C-12), 40.39 (C-8), 31.95 (C-10), 31.26 (C-11) ppm. MALDI-TOF: *m/z* $[M + Na]^+$ (*M* = 1084.46 calculated for $C_{49}H_{72}N_4O_{23}$). ESI-MS: *m/z* = 1107.39 $[M + Na^+]$ (*M* = 1107.45 calculated for $C_{49}H_{72}N_4O_{23}$ + Na).

$(ManC_2)_3C_{11}SAc$ (28): The protected cluster **27** (80 mg, 0.093 mmol) was dissolved in 1.5 mL of DMF at room temp. under nitrogen, 0.3 mL morpholine was added and the reaction mixture was stirred for 2 h. The solvents were removed in vacuo and a colourless solid was obtained and used without further purification. The coupling reaction was carried out according to the general procedure A using acid **12** (22 mg, 0.0843 mmol) in 0.5 mL of DMF, tripropylamine (32 μL, 0.169 mmol) and isobutyl chloroformate (11 μL, 0.0927 mmol) at 0 °C. After 30 min the previously deprotected amine in 1 mL of DMF and 16 μL of NPr_3 was added and the reaction mixture was warmed to room temp. and stirred for 90 h. The solvent was removed in vacuo, the residue was taken up in 15% acetonitrile (aq.) and purified by HPLC chromatography (A = water, B = acetonitrile, 15% B → 70% B, 60 min, 10 mL/min, t_R = 22.73 min) to yield a white foam. Yield 30 mg (0.027 mmol, *M* = 1104.52) 32%. ^1H NMR (500 MHz, MeOD): δ = 4.76 (d, *J* = 1.65 Hz, 3 H, 3 H-1), 3.85 (dd, *J* = 2.2, *J* = 11.74 Hz, 3 H, 3 H-6), 3.82 (dd, *J* = 1.65, 3.3 Hz, 3 H, 3 H-2), 3.76 (m_c, 3 H, 3 H-7), 3.70 (m_c, 6 H, 3 H-3, 3 H-6′), 3.59 (dd, *J* = 9.35, *J* = 9.72 Hz, 3 H, 3 H-4), 3.55 (m_c, 6 H, 3 H-5, 3 H-7′), 3.43 (m_c, 6 H, 3 H-8), 3.37 (m_c, 6 H, 3 H-8′), 2.85 (t, *J* = 7.20 Hz, 2 H, 2 H-23), 2.30 (s, 3 H, 3 H-25), 2.18 (m_c, 8 H, 6 H-10, 2 H-14), 1.98 (m_c, 6 H, 6 H-11), 1.60 (m_c, 2 H, 2 H-15), 1.55 (m, 2 H, 2 H-22), 1.30 [br., 12 H, 2 (H-16, H-17, H-18, H-19, H-20, H-21)] ppm. ^{13}C NMR (125.47 MHz, MeOD): δ = 197.77 (C-24), 176. 06 (C-9), 176.00 (C-13), 101.63 (C-1), 74.81 (C-5), 72.50 (C-3), 72.05 (C-2), 68.66 (C-4), 67.21 (C-7), 63.00 (C-6), 58.89 (C-12), 40.38 (C-8), 37.86 (C-14), 31.79 (C-11), 31.30 (C-10), 30.74 (C-22), 30.49 (C-25), 30.42−29.84 (C-16, C-17, C-18, C-19, C-20. C-21), 29.78 (C-23), 27.15 (C-15) ppm. MALDI-TOF: *m/z* = 1127.83 $[M + Na]^+$ (*M* = 1104.52 calculated for $C_{47}H_{84}N_4O_{23}S$). ESI-MS: *m/z* = 1127.46 $[M + Na^+]$ (*M* = 1127.51 calculated for $C_{47}H_{84}N_4O_{23}S$ + Na).

$(ManC_2)_3sucEG_6C_{11}SAc$ (29): The reaction was carried out according to procedure A but with DIPEA instead of NPr_3 and carboxylic acid **15** (45.5 mg, 0.0745 mmol) in 1.5 mL of DMF, isobutyl chloroformate (11.6 μL, 0.0894 mmol) and DIPEA (28.4 μL, 0.149 mmol). After 30 min of stirring amine **10** (77 mg, 0.0894 mmol) and 14.2 μL of DIPEA were added. This mixture was stirred for 2 h prior to solvent removal in vacuo. The crude product was purified by gel-permeation chromatography on Sephadex LH-20 to yield a colourless oil. Yield: 20.4 mg (0.014 mmol, *M* = 1455.65) 19%. ^1H NMR (500 MHz, MeOD): δ = 4.77 (m, 3 H, 3 H-1), 4.24 (m_c, 2 H, 2 H-17), 3.85 (dd, *J* = 2.1, 11.5 Hz, 3 H, 3 H-6), 3.82 (dd, *J* = 1.6, 3.3 Hz, 3 H, 3 H-2), 3.77 (m_c, 3 H, 3 H-7), 3.70 (m_c, 8 H, 3 H-3, 3 H-6′, 2 H-18), 3.64 [m_c, 18 H, 2 (H-19, H-20, H-21, H-22, H-23, H-24, H-25, H-26, H-27)], 3.64 (m_c, 3 H, 3 H-4), 3.59 (m_c, 3 H, 3 H-5), 3.55 (m_c, 5 H, 3 H-7′, 2 H-28), 3.46 (t, *J* = 6.6 Hz, 2 H, 2 H-29), 3.40 (m_c, 6 H, 6 H-8), 2.86 (t, *J* = 7.34 Hz, 2 H, 2 H-39), 2.67 (m_c, 2 H, 2 H-15), 2.50 (m_c, 2 H, 2 H-14), 2.30 (s, 3 H, 3 H-41), 2.21 (m_c, 6 H, 6 H-10), 1.97 (m_c, 6 H, 6 H-11), 1.55 (m_c, 4 H, 2 H-38, 2 H-30), 1.30 [m_c, 14 H, 2 (H-31, H-32, H-33, H-34, H-35, H-36, H-37)] ppm. ^{13}C NMR (125.47 MHz, MeOD): δ = 197.64 (C-40), 176.06 (C-9), 175.04 (C-13), 173.78 (C-16), 101.69 (C-1), 74.82 (C-5), 72.54 (C-3), 72.37 (C-18), 72.06 (C-2), 71.52, 71.12, 70.04, 68.72 (3 C-4), 67.26 (3 C-7), 65.07 (C-17), 62.98 (C-6), 59.09 (C-12), 40.39 (C-8), 31.81 (C-11), 31.62 (C-15), 31.33 (C-10), 30.74, 30.71 (C-38, C-30), 30.68, 30.59, 30.56, 30.18 (C-41), 29.85 (C-14), 29.77 (C-39), 27.19. ESI-MS: *m/z* = 1477.70 $[M + Na^+]$ (*M* = 1477.71 calculated for $C_{63}H_{114}N_4O_{31}S$ + Na).

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[26] Measured by integration of the NMR signals.

[27] Abbreviations: EDC = 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride, HBTU = *o*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate, HATU = *O*-(7-azabenzotriazol(-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate, SAM = self-assembled monolayer, C_2 = ethyl spacer; C_5 = pentyl spacer; C_{11} = undecyl spacer, EG_6 = hexaethylene glycol spacer, Fuc = β-L-fucoside, Man = α-D-mannoside, suc = succinic acid spacer.

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