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Multivalent glycomimetics: synthesis of nonavalent mannoside clusters with variation of spacer properties

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Dedicated to Manish in appreciation of him joining Anu and Kashi

Abstract—Oligosaccharide mimetics are important tools in the glycosciences. In this work, we have employed spaced glycodendrons for the synthesis of oligomannoside mimetics. Starting from a number of trivalent, branched molecular wedges, the preparation of nonavalent cluster mannosides was accomplished, which were varied with regard to the chemical characteristics of their spacer moieties and spacer lengths. For ligation of the various trivalent dendrons to the nonavalent target molecules peptide coupling was employed.

Keywords: Oligosaccharide mimetics; Cluster mannosides; Spaced glycosides; Peptide coupling

1. Introduction

Curiosity about the biological function of the glycocalyx is one of the major incentives for our synthetic work. The glycocalyx is a fascinating macromolecular system covering every eukaryotic cell.[1] It consists of glycoconjugates of different kinds, which are partly embedded into the plasma membrane via their non-carbohydrate moiety or are attached to the sticky carbohydrate surface of the cell.[2,3] Apparently, many biological functions and important communication processes in cell life can be associated with the properties and features of the cell surface glycocalyx.[4] Important tools to investigate the biological function of the glycocalyx are synthetic glycoconjugates that resemble portions of the structures found in the glycocalyx. The design of such molecules is inspired by the architecture of the natural example structures on one hand; on the other hand artificial architectures have also led to valuable molecules.[5–9] Such synthetic glycoconjugates have been shown to be especially useful for glycobiological studies, when they allow multimerization of the essential carbohydrate moieties, as well as the variation of their characteristics with regard to their spacial arrangement and conformational degree of freedom,[10,11] as in the case of glycodendrimers, glycodendrons, and glycoclusters, respectively.[12–17]

An important scenario investigated in glycobiology, which depends on multivalent carbohydrate–protein interactions, is the colonization of cell surfaces by bacteria such as *Escherichia coli*. Whereas colonization by *E. coli* causes no problem in the intestine, the urogenital tract suffers from *E. coli* infections, responding with inflammation,[18] or apoptosis.[19] Bacteria accomplish adhesion to their host cells using organelles exposed on their own surface, which are called fimbriae or pili.[20] It is of great biological and medicinal interest to understand the mechanisms that are associated with adhesion of bacteria and its consequences for the physiology of the affected cells. The so-called type 1 fimbriae possess lectin domains, resembled by a protein named FimH,[21] which is specific for α-mannosyl residues.[22] To gain more knowledge about the biochemical consequences of mannose-specific bacterial adhesion, it became our

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goal to synthesize high-molecular weight glycoconjugates exposing differently spaced mannose residues.

For the design of our target molecules we took advantage of the synthesis of branched trivalent cluster mannosides, which we have reported earlier.[23] This type of cluster mannoside carries a nitro group at the focal point of the scaffold, which was planned to serve as masked amino function, to eventually allow multimerization of the respective molecular wedge by peptide coupling to a branched oligo-carboxylic acid.[24] A requirement for the selected synthetic strategy was the possibility to vary the properties of the spacer moieties integrated between the glycocluster head groups of the target molecules and their central branched core. Consequently, we started two synthetic routes as outlined in Figure 1. In route 1, the core molecule is first function-

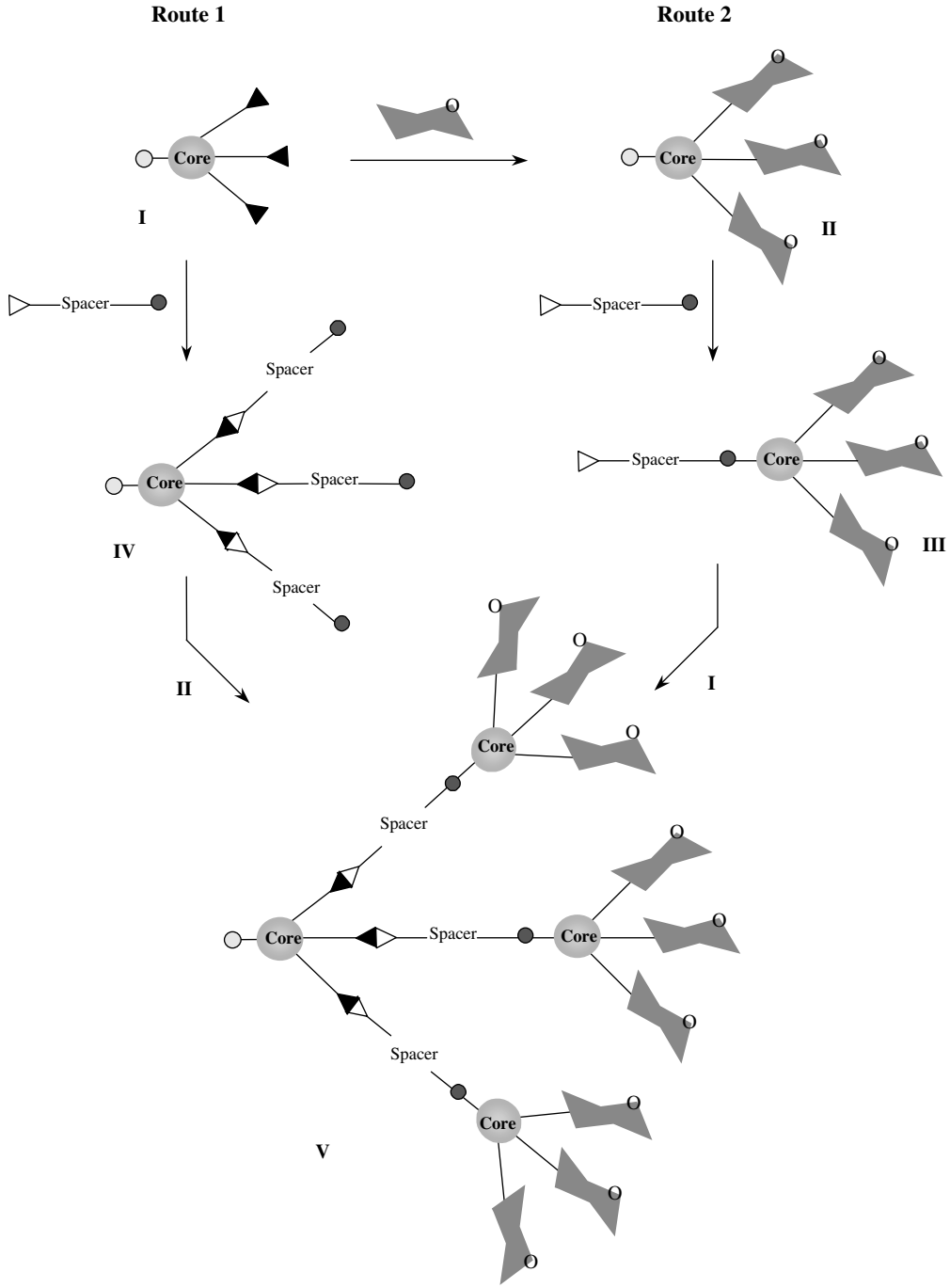


Figure 1. Glycocoating of an underivatized branched core molecule **I** can lead to glycoclusters of type **II**, which can be multimerized through different spacers, following two routes. Route 1: Spacer derivatization of core **I** leads to spaced core **IV** first, onto which glycocluster **II** can be anchored in the next step leading to its multivalent form **V**. Route 2: First, a spacer is attached to the focal point of glycocluster **II** giving a spaced glycocluster of type **III**. This can then be clustered onto a multivalent core **I** to again furnish its multivalent form **V**.

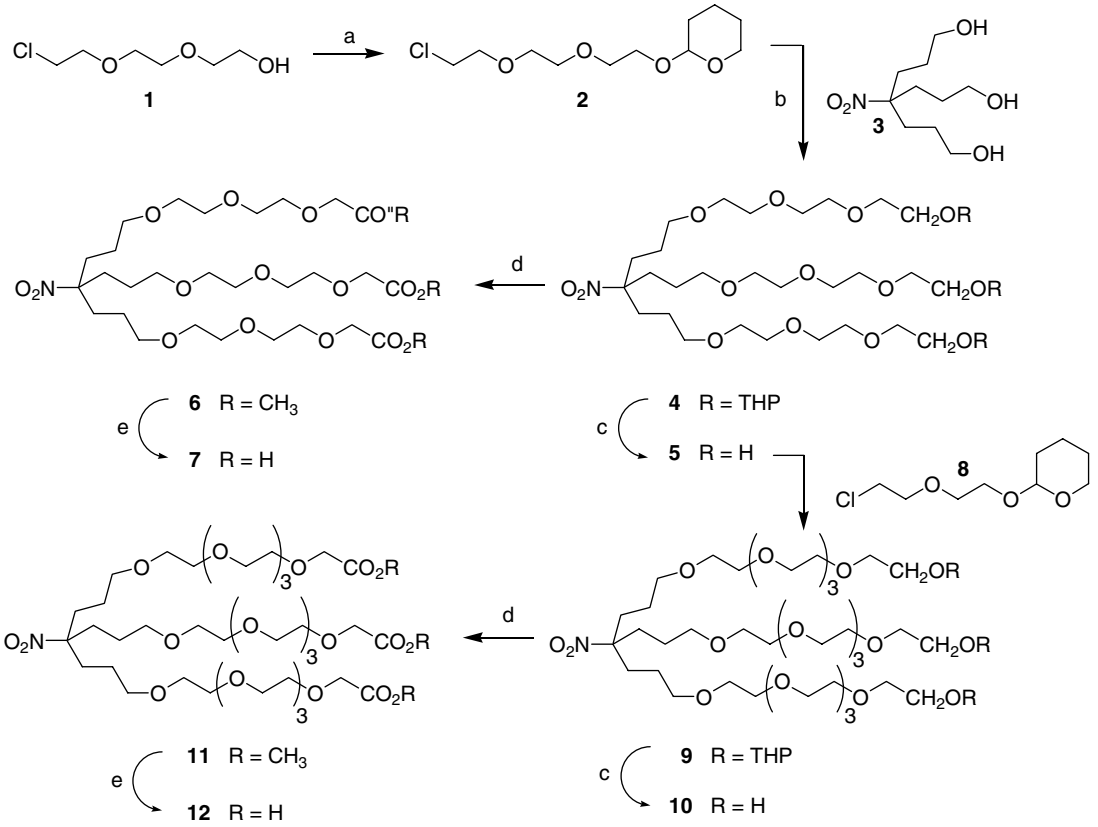
alized with different spacers and then the glycocluster head groups are attached to the periphery of the spaced trivalent core. In route 2, the cluster mannoside is prepared first, activated and spaced at its focal point and multimerized afterwards using an underivatized branched tricarboxylic acid core.

2. Results and discussion

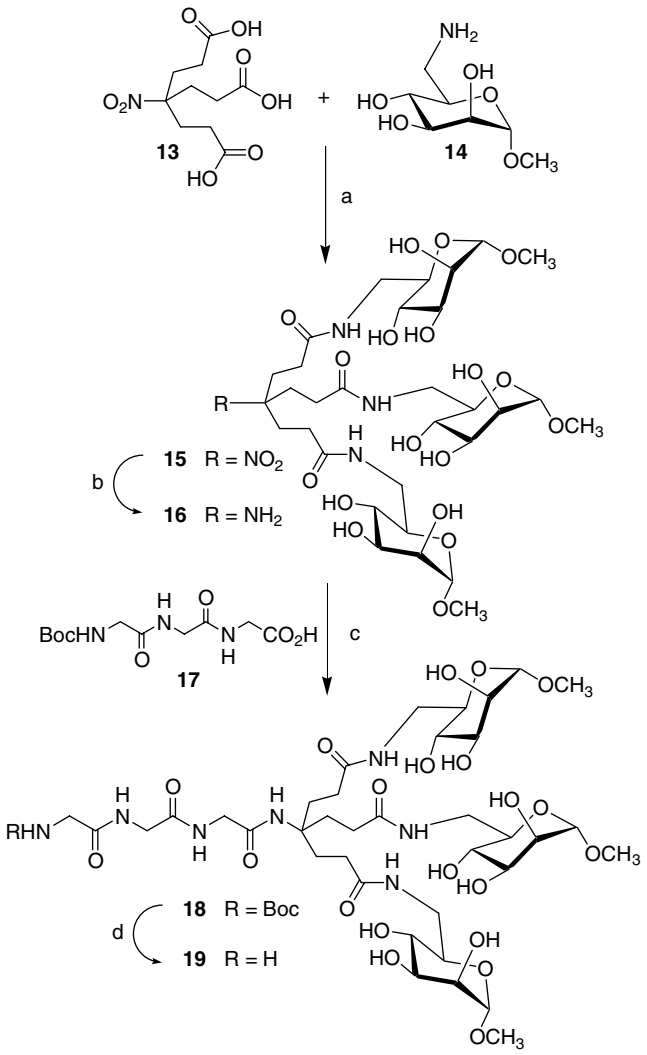
As explained in the introduction and depicted in Figure 1 it was the goal of this work to prepare differently spaced nonavalent mannoside clusters. Three different spacers were chosen, two of the oligoethylene glycol-type (**7** and **12** in Scheme 1) and one of a peptide-type (**17** in Scheme 2). As the central core molecule (Fig. 1, core I) the branched triol 4-nitro-4-(3-hydroxypropyl)-heptan-1,7-diol (**3**)[25] and the respective tricarboxylic acid **13**[25] were selected. As the glycocluster head group (Fig. 1, cluster II) the known trivalent cluster mannoside **15**[23] was employed and further modified. Finally, trimesic acid (**23**) also was used as core for multimerization.

2.1. Synthesis of spaced dendrons

At first, following route 1 in Figure 1, the oligoethylene glycol-spaced tricarboxylic acids **7** and **12** (Scheme 1) had to be prepared as trivalent branched core molecules. Their synthesis began with protection of 2-[2-(2-chloroethoxy)-ethoxy]ethanol (**1**) with dihydropyran in the presence of a catalytic amount of *p*-toluenesulfonic acid. The expected THP-protected alkyl chloride **2** was obtained as racemate and reacted with the branched nitro-triol **3**[25] in an etherification reaction using aqueous NaOH solution under phase transfer catalysis. The ethylene glycol-spaced product **4** carrying three terminal tetrahydropyran groups was isolated in 68% yield. Its structure was confirmed by ^{1}H and ^{13}C NMR spectra where characteristic signals corresponding to the CH protons of the THP ring (4.63 ppm) and the peak of the quaternary carbon atom at the focal point of the molecule (95.1 ppm) were indicative. Additional confirmation of the structure was obtained by MALDI-TOFMS showing the $[M]^{+}$ peak at $m/z = 883.7$. Then, **4** was deprotected to triol **5** in good yield using methanolic-HCl solution, followed by oxidation of the hydroxyl



Scheme 1. Synthesis of polyethylene glycol-spaced tricarboxylic acids **7** and **12**. Reagents and conditions: (a) dihydropyran, CH_2Cl_2, *p*-TsOH, 0 °C→rt, 1 h, 83%; (b) aq NaOH, *n*-Bu_4NBr, 80 °C, 12 h, 68%; (c) HCl–MeOH (10%), 0 °C, 1 h, 77% for **5**, 79% for **10**; (d) 1. TEMPO, NaOCl, KBr, *n*-Bu_4NCl, satd $NaHCO_3$, brine, CH_2Cl_2, 0 °C→rt, 1 h; 2. DOWEX 50W × 2, MeOH, rt, 1 h, 50% for **6**, 49% for **11** over two steps; (e) $LiOH{\cdot}H_2O$, $MeOH/H_2O$ (1:2), 0 °C, quant.



Scheme 2. Synthesis of functionalized trivalent cluster mannosides **16** and **19**. Reagents and conditions: (a) HATU, DIPEA, DMF, rt, 6 h, 92%; (b) ammonium formate, Pd–C, H_2, MeOH, rt, 24 h, 79%; (c) HATU, DIPEA, DMF, rt→45 °C, 12 h, 67%; (d) dimethyl sulfide/CF_3COOH (1:2), 0 °C, 3 h, 86%.

groups with TEMPO.[26] To facilitate the purification of the product, the tricarboxylic acid resulting from TEMPO oxidation was subjected to an esterification reaction using acidic ion exchange resin in methanol, which led to triester **6**. The product was obtained in 50% overall yield from **5** and its structure was confirmed by NMR and MALDI-TOF mass spectroscopy. After purification, triester **6** was subjected to base-catalyzed ester hydrolysis using LiOH to obtain the corresponding triacid **7**, which was used later without further purification.

For the synthesis of a trivalent core molecule with homologous, however elongated spacers, triol **5** was subjected to etherification with 2-(2-chloroethoxy)-*O*-tetrahydropyranyl ethanol (**8**) to yield the protected triol **9**. By analogy to the synthetic pathway followed for the synthesis of **7**, **9** was deprotected to triol **10**, which was oxidized using TEMPO followed by resin-catalyzed esterification of the oxidation product to give the branched triester **11**. Hydrolysis with lithium hydroxide later afforded the desired triacid derivative **12**.

2.2. Synthesis of the trivalent mannoside head groups

As the clustered glycosidic head group of all target molecules, the trivalent cluster mannoside **16** was needed. This glycocluster has been synthesized earlier in our group by peptide coupling of the 6-amino-6-deoxy mannoside **14** and the branched tricarboxylic acid **13**.[23] This coupling reaction activated by HATU[27] delivered the target molecule **15** in an improved yield of 92% as compared to the literature. In the past, it has been a problem to reduce the nitro group at the focal point of cluster mannoside **15**,[28] which is necessary to allow further functionalization and multimerization of the molecule. When **15** was subjected to hydrogenation with activated Raney-nickel, reduction was not successful even under drastic reaction conditions; other reducing agents such as lithium aluminum hydride and BH_3–THF failed as well. Also, when ammonium formate was employed as hydrogen transfer reagent in a palladium-catalyzed reaction,[29] reduction could not be accomplished. However, with a combination of an excess of ammonium formate, a catalytic amount of activated palladium and hydrogen under atmospheric pressure employed, direct reduction of **15** to the desired amine **16** succeeded. This procedure delivered cluster mannoside **16** in 79% yield (Scheme 2).

Proof for the complete reduction of the nitro group to the corresponding amine was obtained by comparison of the ^{13}C NMR spectral data, where a drastic upfield shift of the peak corresponding to the quaternary core carbon from 93.3 ppm (**15**) to 52.7 ppm (**16**) was detected. Also, the ^1H NMR spectrum was indicative as in the spectrum of **16** splitting of the signal for the ethyl spacer protons into two different sets was found, resonating separately at 2.19 and 1.60 ppm, whereas in **15** the protons of both methylene groups resonated together at 2.25 ppm. Additional confirmation of the successful reduction was obtained by the MALDI-TOF mass spectrum of **16**, which showed the accurate mass peak at $m/z = 773.3$ for $[M+H]^+$.

In addition to the implementation of oligoethylene glycol-type spacers, it was our goal to access also nonavalent cluster mannosides with spacer moieties of the peptide type. As depicted in Figure 1, we followed a route where the peptide portion was attached to the focal point of the trivalent cluster mannoside **16** rather than to elongate the trivalent nitro triol **3**.

The tripeptide triglycine was selected as the spacer (Scheme 2) and treated with di-*tert*-butyl dicarbonate in a solution of aqueous $NaHCO_3$/DMF (4:3), which afforded the corresponding *N*-Boc-protected derivative **17** in 96% yield. Protection of the amino group was con-

firmed by ^{1}H NMR spectroscopy, where the characteristic singlet corresponding to the *tert*-butyl protons (1.42 ppm) appeared. The HATU-mediated coupling reaction between the *N*-Boc-protected triglycine **17** and the amino-functionalized cluster mannoside **16** gave the desired triglycine-spaced cluster mannoside **18** in 67% yield, with a $[M+Na]^{+}$ peak at $m/z = 1066.5$ in the MALDI-TOFMS. Prolonged reaction times in the peptide coupling step (24 instead of 12 h) were necessary to achieve acceptable yields. Removal of the Boc group was accomplished using an anhydrous mixture of trifluoroacetic acid and dimethyl sulfide (2:1)[30] leading to the free amine **19** in 86% yield.

2.3. Synthesis of nonavalent mannoside clusters

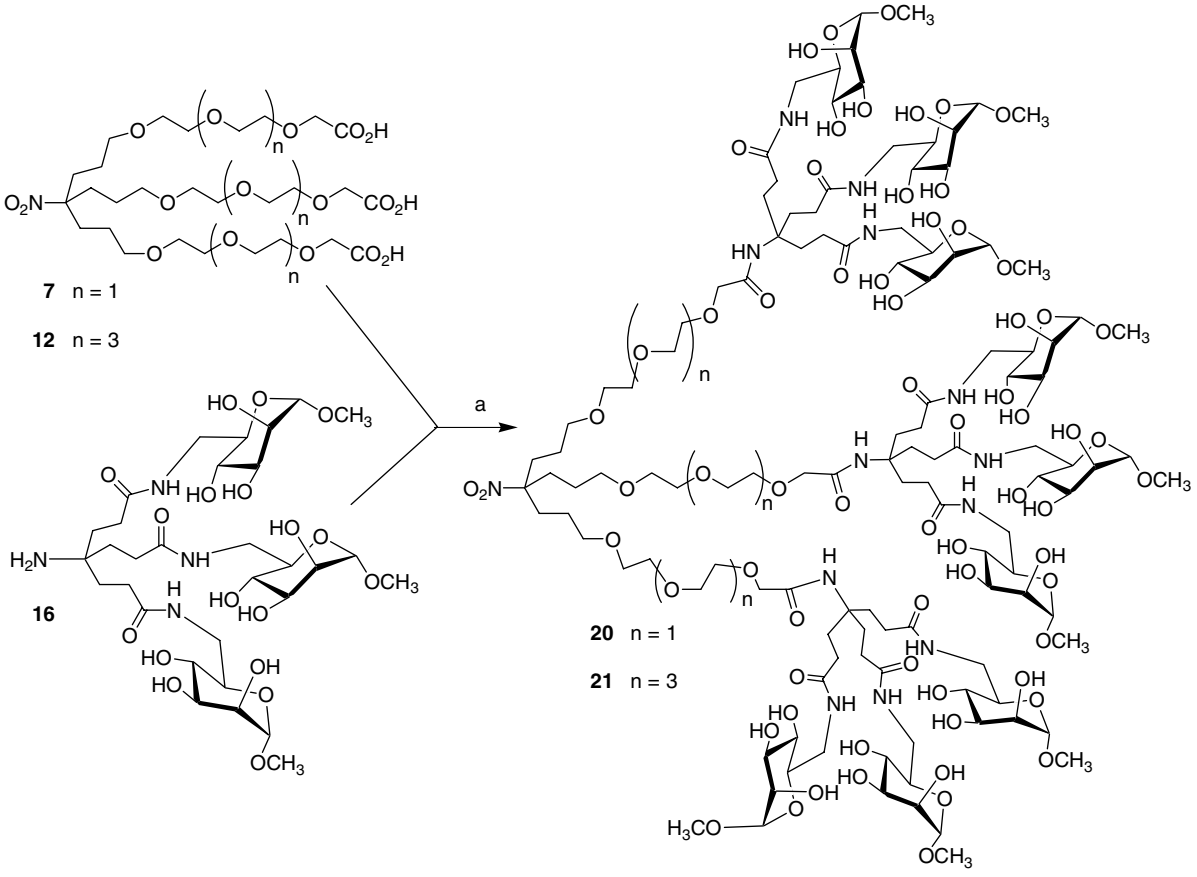
To multimerize the trivalent glycocluster head groups, first peptide coupling of the amino-functionalized cluster mannoside **16** and the branched oligoethylene glycol-spaced tricarboxylic acids **7** and **12** was attempted (Scheme 3). The HATU-activated reaction of **16** with **7** led to dendron **20** in approximately 40%, bearing nine mannosyl residues and a nitro group at the focal point, masking an amino function for further modifications. The ^{1}H and ^{13}C NMR spectra of **20** showed signals in the ^{13}C NMR spectrum corresponding to both coupling units **7** and **16**, such as the quaternary core carbon atom adjacent to the nitro group (95.1 ppm) and those quaternary core carbon atoms connected to the peptide bond (60.6 ppm). The monodisperse character of dendron **20** was confirmed by the MALDI-TOFMS.

Employing the elongated branched tricarboxylic acid **12** in an analogous HATU-mediated peptide coupling reaction with cluster mannoside **16** afforded the extended glycodendron **21** in 38% yield.

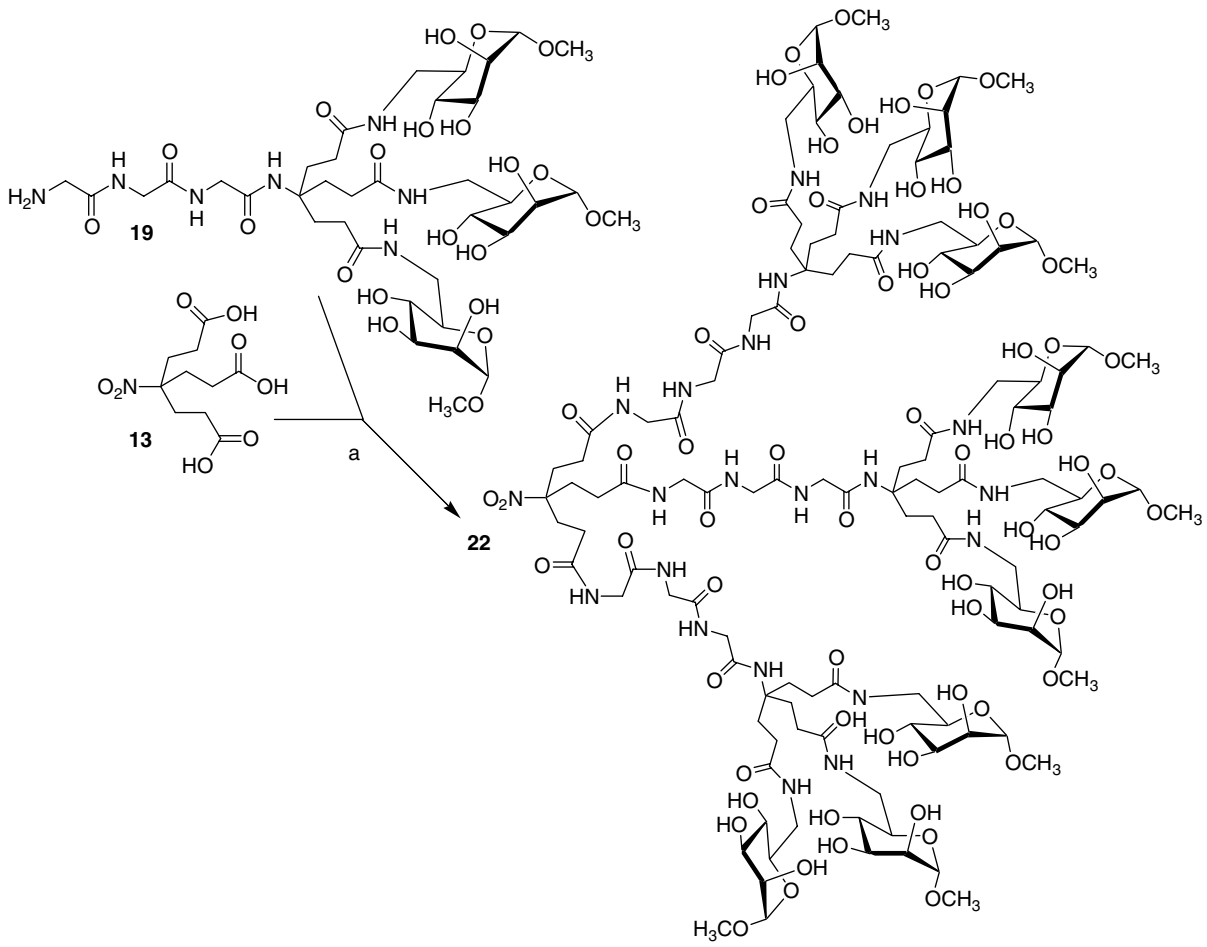
Finally, the triglycine-spaced nonavalent glycocluster **22** was obtained by coupling the peptide-modified trivalent cluster mannoside **19** and the branched tricarboxylic acid **13** (Scheme 4). By analogy, **19** could be multimerized on the basis of trimesic acid (Scheme 5) leading to **24**. Applying the reliable HATU protocol, the two high-molecular weight glycodendrons, **22** and **24**, were obtained in 29% and 33% yield, respectively, after extensive purification. In addition to advanced NMR spectroscopy, the structures were confirmed by mass spectrometry.

3. Biological testing and conclusions

When the prepared nonavalent cluster mannosides were tested as inhibitors of the type 1 fimbriae-mediated



Scheme 3. Reagents and conditions: (a) HATU, DIPEA, DMF, rt→45 °C, 12 h, 41% for **20**; 38% for **21**.



Scheme 4. Reagents and conditions: (a) HATU, DIPEA, DMF, rt→45 °C, 12 h, 29%.

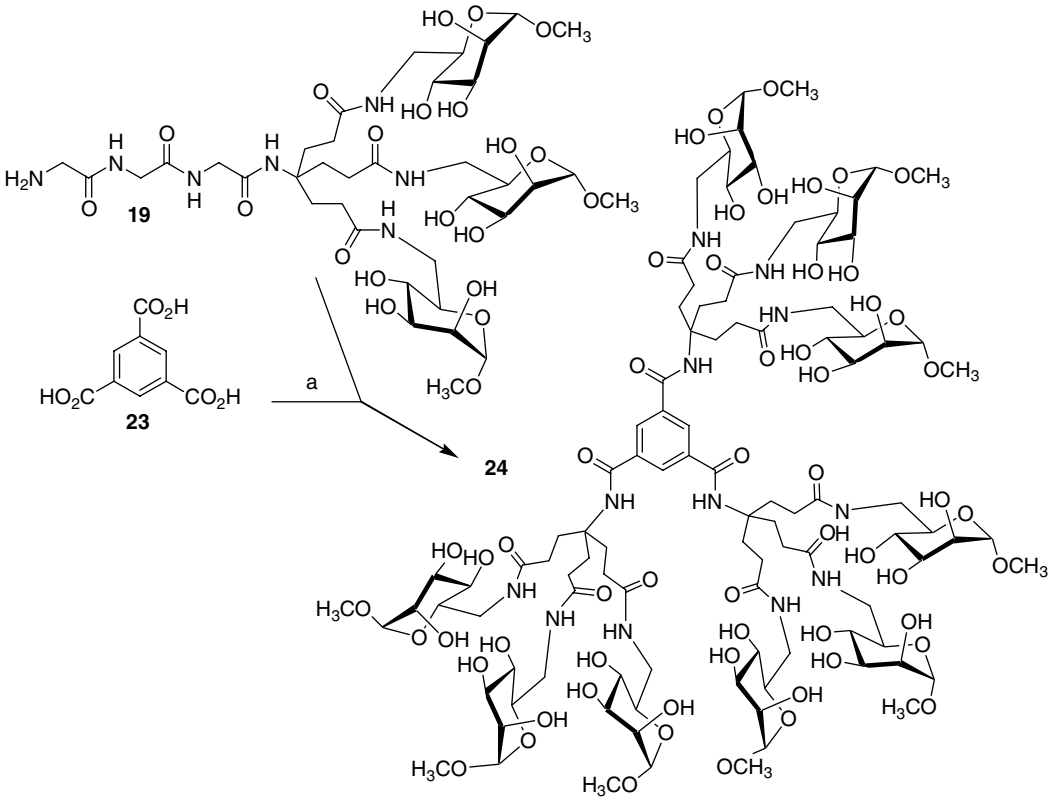
adhesion, using an appropriate ELISA as reported earlier,[28] surprisingly only very poor or no inhibitory potencies of these multivalent glycoconjugates were found. This finding was confirmed by several independent measurements. There are several possible explanations and interpretations for the fact that the nonavalent cluster mannosides reported here do not inhibit type 1 fimbriae-mediated bacterial adhesion. It has been reported in the literature that minor impurities can cause false positive results in binding assays, as for example, impurities from ion exchange resin, which have led to extremely potent inhibition of selectin binding.[31] However, it is less likely that impurities lead to deletion of binding potency. More probably, the prepared glycoconjugates do not present enough hydroxyl groups for the interaction with type 1 fimbriae, or do not present hydroxyl or other functional groups in the appropriate orientation for lectin binding.

Future work will address the lack of anti-adhesive properties of the nonavalent cluster mannosides reported here in a biological context. We will be able to take advantage of the modular approach to multivalent glycoconjugates, which has been elaborated in this work. We have shown that branched trivalent cluster mannosides with a masked amino function at their focal point are suitable molecular wedges to be assembled on multivalent core molecules. This approach, utilizing strategies of dendrimer chemistry, also facilitates the implementation of spacer moieties and their variation with regard to parameters such as hydrophilicity and molecular flexibility.

4. Experimental

4.1. General methods

TLC and flash chromatography were performed on silica gel 60 (230–400 mesh, 40–63 μm, Merck) and detection was carried out by charring with 20% ethanolic sulfuric acid solution containing 5% of α-naphthol and under UV light when applicable. For size exclusion chromatography, Sephadex LH-20 was used with MeOH as the eluent and Bio-gel P-2 and Bio-gel P-6



Scheme 5. Reagents and conditions: (a) HATU, DIPEA, DMF, rt→45 °C, 12 h, 33%.

with 15 mM aq NH_4HCO_3 buffer (pH = 7.8–8.0) as the eluent. Organic solutions were concentrated using a rotary evaporator at bath temperatures <45 °C. Aqueous solutions were concentrated by lyophilization. ^1H and ^{13}C NMR spectra were recorded at 298 K, at 300 MHz (for ^1H, 75.46 MHz for ^{13}C NMR), 400 MHz (for ^1H, 100.67 MHz for ^{13}C NMR) and 500 MHz (for ^1H, 125.84 MHz for ^{13}C NMR). Chemical shifts are given in parts per million relative to internal TMS (0.00 ppm for ^1H and ^{13}C NMR) and when the samples were measured in D_2O the spectra were calibrated referenced to internal HOD (4.63 ppm for ^1H NMR) and in the case of ^{13}C NMR to [d_4]-MeOH (49.30 ppm for ^{13}C NMR), which was added to the solution. Coupling constant (J) values are given in hertz. Two-dimensional ^1H–^1H and ^1H–^{13}C COSY (HMQC) experiments were performed for complete signal assignments wherever necessary. IR spectra were recorded on FT-IR ATI Mattson Instruments (USA). MALDI-TOFMS were recorded on Bruker Biflex and ESI-MS on Finnigan MAT 95. For MALDI-TOF measurements, the samples were prepared as solutions in acetonitrile/water/TFA, 2:1:0.1, with a concentration of 1 mg/mL solution. Compounds were co-crystallized with either 2,5-dihydroxy benzoic acid (DHB) or α-cyano-4-hydroxycinnamic acid (CCA). The mass peaks obtained with all the samples were calibrated in reference to the $[M+H]^+$ peaks of angiotensin II (1046.54), angiotensin I (1296.69), bombesin (1619.82), and to the $[2M+H]^+$ peak of CCA (380.02). High resolution ESI mass spectra were measured with an Applied Biosystems Mariner ESI-TOF 5280. Optical rotations were recorded at Na-D line, 589 nm, 20 °C, cell length 10 and 1 cm in special cases. Elemental analyses were carried out at the Institute of Inorganic Chemistry, Christiana Albertina University of Kiel. Triglycine, trimesic acid (**23**), DOWEX 50W × 2 (H^+, 100–200 mesh) and HATU were purchased from Fluka. 4-Nitro-4-(-3-hydroxypropyl)-heptan-1,7-diol (**3**), 2-(2-chloroethoxy)-ethanol, and 2-(2-{2-chloroethoxy}-ethoxy)-ethanol (**1**) were purchased from Aldrich.

4.2. Synthesis of polyethylene glycol-spaced tricarboxylic acids

4.2.1. 2-{2-(2-Chloroethoxy)-ethoxy}tetrahydropyranyl-ethanol (2). To an ice-cold solution of 2-{2-(2-chloroethoxy)-ethoxy}-ethanol **1** (2.0 g, 11.86 mmol) and dihydropyran (1.6 mL, 17.79 mmol) in dry CH_2Cl_2 (20 mL), *p*-TsOH (50 mg) was added. The reaction mixture was warmed to rt and further stirred for 1 h. It was then neutralized with Et_3N, concentrated, and the crude product was purified by flash chromatography (*n*-hexane/EtOAc, 4:1) to give the title compound (2.50 g,

9.90 mmol, 83%) as a colorless syrup; $R_f = 0.3$ (4:1 hexane/EtOAc, UV, α-naphthol); ^{1}H NMR (300 MHz, [d_6]-acetone): δ 4.63 (m_c, 1H, OCHO), 3.80–3.66 (m, 6H, CH_2Cl, $2OCH_2$), 3.65–3.60 (m, 6H, $3OCH_2$), 3.56–3.39 (m, 2H, ring-OCH_2), 1.86–1.44 (m, 6H, $3CH_2$); ^{13}C NMR (75.46 MHz, [d_6]-acetone): δ 99.2 (OCHO), 72.0, 71.3, 71.2, 71.1, 67.2, 62.1 ($6OCH_2$), 44.0 (CH_2Cl), 31.3, 26.3, 20.0 ($3CH_2$); ESIMS: $m/z = 253.1$ $[M+H]^+$ observed for $C_{11}H_{21}O_4Cl$ (252.11).

4.2.2. Tris-[3-{2-(bis-[2-ethoxy])tetrahydropyranylethoxy}-propyloxy]nitromethane (4). 4-Nitro-4-(3-hydroxypropyl)-heptan-1,7-diol (**3**) (0.10 g, 0.42 mmol) was suspended in NaOH solution (5 mL, 1 g/mL in H_2O) and warmed to 80 °C for 30 min. To the homogeneous reaction mixture, the alkyl chloride **2** (0.43 g, 1.70 mmol) was added, followed by the addition of *n*-Bu_4NBr (13 mg, 0.04 mmol). The reaction mixture was further stirred for 12 h at 80 °C. Then it was cooled to rt and diluted with water. The product was extracted with diethyl ether (10 mL) and the ether layer was successively washed with H_2O and brine. After drying over anhyd Na_2SO_4 it was filtered, concentrated, and the crude product was purified by flash chromatography (1.5:1 hexane/acetone) to afford the tris-THP derivative **4** (0.26 g, 0.29 mmol, 68%) as a colorless syrup; $R_f = 0.2$ (*n*-hexane/acetone, 1.5:1; UV, α-naphthol). ^{1}H NMR (500 MHz, [d_6]-acetone): δ 4.63 (m_c, 3H, 3OCHO), 3.85–3.76 (m, 6H, 3 ring-OCH_2), 3.64–3.58 (m, 26H, $13OCH_2$), 3.56–3.51 (m, 8H, $4OCH_2$), 3.50–3.42 (m, 8H, $4OCH_2$), 2.07–2.01 (m, 6H, $3CH_2$), 1.85–1.75 (m, 3H, 3 ring-CH), 1.69–1.61 (m, 3H, 3 ring-CH), 1.57–1.42 (m, 18H, 6 ring-CH_2, $3CH_2$); ^{13}C NMR (125.75 MHz, [d_6]-acetone): δ 99.2 (3OCHO), 95.1 (NO_2–C_q), 71.3, 71.2, 71.1, 71.0, 70.9, 70.8, 67.2, 62.0 ($24OCH_2$), 32.9 ($3CH_2$), 31.3, 26.2 (6 ring-CH_2), 24.7 ($3CH_2$), 20.0 (3 ring-CH_2); MALDI-TOFMS: $m/z = 867.5$ $[M-16]^+$, 883.7 $[M]^+$, 907.1 $[M+Na]^+$, and 923.1 $[M+K]^+$ observed for $C_{43}H_{81}NO_{17}$ (883.55).

4.2.3. Tris-[3-{2-(bis-[2-ethoxy])-ethanoyl}-propyloxy]-nitromethane (5). A solution of the tris-THP derivative **4** (100 mg, 110 μmol) in MeOH (1 mL) was treated with HCl solution (10% in MeOH, 0.5 mL) at 0 °C for 1 h. The acidic reaction mixture was quenched by the addition of aq $NaHCO_3$ solution and concentrated. The crude product was purified by flash chromatography (1:9 MeOH/CH_2Cl_2) to afford the deprotected triol **5** (54 mg, 85.0 μmol, 77%) as a colorless syrup; $R_f = 0.4$ (MeOH/CH_2Cl_2, 1:9; α-naphthol); ^{1}H NMR (500 MHz, D_2O): δ 3.81–3.73 (m, 24H, $12OCH_2$), 3.72–3.69 (m, 12H, $3OCH_2$, $3CH_2OH$), 3.64–3.61 (t, $J = 6.36$ Hz, 6H, $3OCH_2$), 2.13–2.07 (m, 6H, $3CH_2$), 1.61–1.54 (m, 6H, $3CH_2$); ^{13}C NMR (125.75 MHz, D_2O): δ 97.7 (NO_2–C_q), 74.3, 72.9, 72.3, 72.2, 72.1, 71.8 ($18OCH_2$), 63.0 ($3CH_2OH$), 34.2 ($3CH_2$), 25.62 ($3CH_2$); MALDI-TOFMS: $m/z = 615.3$ $[M-16]^+$, 631.3 $[M]^+$, 654.5 $[M+Na]^+$, and 670.5 $[M+K]^+$ observed for $C_{28}H_{57}NO_{14}$ (631.37).

4.2.4. Tris-[3-{2-(bis-[2-ethoxy])methoxycarbonyl}propyl-oxy]nitromethane (6). To a mixture of triol **5** (54 mg, 85.0 μmol) and TEMPO (0.5 mg, 3.2 μmol) in CH_2Cl_2 (0.5 mL), a solution of satd $NaHCO_3$ (0.6 mL) containing KBr (3.1 mg, 25.0 μmol) and *n*-Bu_4NCl (4.0 mg, 14.0 μmol) was added. Then a solution of NaOCl (13%, 0.7 mL), $NaHCO_3$ (0.3 mL), and brine (0.6 mL) was successively added at 0 °C over 30 min. The reaction mixture was allowed to attain rt and further stirred for 1 h. Then the organic layer was separated from aqueous layer and the aqueous phase was acidified (pH = 6.0) with HCl (2 N) at 0 °C and lyophilized to give the triacid derivative **7** as a white lyophilisate. DOWEX 50W × 2 (H^+, 100–200 mesh, 50 mg) was added to a suspension of **7** in MeOH and the mixture was stirred at rt for 1 h. The solid suspension was filtered and the filtrate was concentrated to provide the crude product, which was purified by flash chromatography (1:1 MeOH/CH_2Cl_2) to afford the triester derivative **6** (30 mg, 42.0 μmol, 50% over two steps) as a colorless syrup; $R_f = 0.7$ (MeOH/CH_2Cl_2, 1:1; α-naphthol); ^{1}H NMR (500 MHz, $CDCl_3$): δ 4.16 (s, 6H, $3OCH_2CO$), 3.73 (s, 9H, $3CO_2CH_3$), 3.73–3.69 (m, 6H, $3OCH_2$), 3.68–3.65 (m, 6H, $3OCH_2$), 3.63–3.59 (m, 6H, $3OCH_2$) 3.56–3.53 (m, 6H, $3OCH_2$), 3.43–3.40 (t, $J = 6.37$ Hz, 6H, $3OCH_2$), 1.96–1.92 (m, 6H, $3CH_2$), 1.49–1.43 (m, 6H, $3CH_2$); ^{13}C NMR (125.75 MHz, $CDCl_3$): δ 170.9 ($3CO_2CH_3$), 94.2 (NO_2–C_q), 70.9 ($3OCH_2$), 70.7, 70.6 ($9OCH_2$), 70.2 ($3OCH_2$), 68.6 ($3OCH_2CO$), 51.8 ($3CO_2CH_3$), 32.2 ($3CH_2$), 24.0 ($3CH_2$); MALDI-TOFMS: $m/z = 699.5$ $[M-16]^+$, 715.4 $[M]^+$, 738.7 $[M+Na]^+$, and 754.7 $[M+K]^+$ observed for $C_{31}H_{57}NO_{17}$ (715.36).

4.2.5. Tris-[3-{2-(tetrakis-{2-ethoxy})tetrahydropyranyl-ethoxy}propyloxy]nitromethane (9). Etherification of triol **5** (0.22 g, 0.35 mmol) with the THP-protected alkyl chloride **8** (which was easily obtained from commercially available 2-(2-chloroethoxy)-ethanol in analogy to the synthesis of **2**; 0.33 g, 1.56 mmol) was accomplished using *n*-Bu_4NBr (11 mg, 0.05 mmol) and NaOH solution (5 mL, 1 g/mL in H_2O) similarly as in the case of compound **4**. The crude product was purified by flash chromatography (19:1 CH_2Cl_2/MeOH) to afford the title compound **9** (0.35 g, 0.30 mmol, 86%) as a colorless syrup; $R_f = 0.5$ (CH_2Cl_2/MeOH, 19:1; UV, α-naphthol); ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.64 (m_c, 3H, 3OCHO), 3.91–3.83 (m, 6H, 3 ring-OCH_2), 3.78–3.51 (m, 60H, $30OCH_2$), 3.42 (t, 6H, $3CH_2CH_2O$), 2.0–1.92 (m, 6H, $3CH_2$), 1.91–1.71 (m, 6H, 3 ring-CH_2), 1.70–1.42 (m, 18H, 6 ring-CH_2, $3CH_2$); ^{13}C NMR (75.46 MHz, $CDCl_3$): δ 98.9 (3OCHO), 94.7 (NO_2–C_q), 72.6, 70.7,

70.6, 70.5, 70.3, 70.2, 70.1, 66.6, 63.0, 62.2, 61.7 ($36OCH_2$), 32.2 ($3CH_2$), 30.7, 30.5 (3 ring-CH_2), 25.4 (3 ring-CH_2), 24.0 ($3CH_2$), 19.8, 19.5 (3 ring-CH_2); MALDI-TOFMS: $m/z = 1131.9$ $[M-16]^+$, 1147.3 $[M]^+$, 1171.0 $[M+Na]^+$, and 1187.0 $[M+K]^+$ observed for $C_{55}H_{105}NO_{23}$ (1147.70).

4.2.6. Tris-[3-{2-(tetrakis-{2-ethoxy})ethanoyl}propyl-oxy]nitromethane (10). THP-deprotection was carried out with **9** (0.32 g, 0.28 mmol) in HCl solution (10% in MeOH, 0.5 mL), following the experimental procedure described for the synthesis of **5**. The crude product was purified by flash chromatography (1:9 MeOH/CH_2Cl_2) to afford the spaced triol **10** (0.20 g, 0.22 mmol, 79%) as a colorless syrup; $R_f = 0.4$ ($MeOH/CH_2Cl_2$, 1:9; α-naphthol); ^1H NMR (300 MHz, 1:1 $CDCl_3/CD_3OD$): δ 3.72–3.64 (m, 48H, $21OCH_2$, $3CH_2OH$), 3.62–3.58 (m, 12H, $6OCH_2$), 3.49 (t, $J = 6.29$ Hz, 6H, $3OCH_2CH_2$), 2.08–1.96 (m, 6H, $3CH_2$), 1.56–1.45 (m, 6H, $3CH_2$); ^{13}C NMR (75.46 MHz, 1:1 $CDCl_3/CD_3OD$): δ 93.7 (NO_2–C_q), 72.0, 70.0, 69.9, 69.8, 69.6, 69.5, 69.4 ($30OCH_2$), 60.6 ($3CH_2OH$), 31.6 ($3CH_2$), 23.4 ($3CH_2$); MALDI-TOFMS: $m/z = 879.7$ $[M-16]^+$, 895.3 $[M]^+$, 918.7 $[M+Na]^+$, and 934.7 $[M+K]^+$ observed for $C_{40}H_{81}NO_{20}$ (895.53).

4.2.7. Tris-[3-{2-(tetrakis-{2-ethoxy})-methoxycarbonyl}-propyloxy]-nitromethane (11). To a solution of triol **10** (200 mg, 223 μmol) in CH_2Cl_2 (1.5 mL), TEMPO (1.2 mg, 7.2 μmol) and a solution of satd $NaHCO_3$ (1.6 mL) containing KBr (8.0 mg, 67.0 μmol) and *n*-Bu_4NCl (10 mg, 36.0 μmol) were added. Then the reaction mixture was cooled to 0 °C and treated with a solution of NaOCl (13%, 1.8 mL), $NaHCO_3$ (0.8 mL), and brine (1.6 mL) similarly as described for compound **6**. This afforded the crude triacid **12** as a white lyophilisate, which was then treated with DOWEX 50W × 2 (H^+, 100–200 mesh, 50 mg) to get the corresponding triester derivative **11**. The crude product was purified by flash chromatography (1:9 $MeOH/CH_2Cl_2$) to afford pure **11** (100 mg, 109 μmol, 49% over two steps) as a colorless syrup; $R_f = 0.6$ ($MeOH/CH_2Cl_2$, 1:9; α-naphthol); ^1H NMR (500 MHz, 1:1 $CDCl_3/CD_3OD$): δ 4.16 (s, 6H, $3OCH_2CO$), 3.73 (s, 9H, $3CO_2CH_3$), 3.75–3.72 (m, 6H, $3OCH_2$), 3.71–3.68 (m, 6H, $3OCH_2$), 3.67–3.63 (m, 30H, $15OCH_2$) 3.61–3.57 (m, 6H, $3OCH_2$), 3.48 (t, $J = 6.29$ Hz, 6H, $3OCH_2CH_2$), 2.02–1.98 (m, 6H, $3CH_2$), 1.53–1.47 (m, 6H, $3CH_2$); ^{13}C NMR (125.75 MHz, 1:1 $CDCl_3/CD_3OD$): δ 171.8 ($3CO_2CH_3$), 94.7 (NO_2–Cq), 71.3 ($3OCH_2$), 71.1, 71.0, 70.9 ($21OCH_2$), 70.6 ($3OCH_2$), 68.8 ($3OCH_2CO$), 52.1 ($3CO_2CH_3$), 32.7 ($3CH_2$), 24.4 ($3CH_2$); MALDI-TOFMS: $m/z = 963.5$ $[M-16]^+$, 980.9 $[M+H]^+$, 1002.5 $[M+Na]^+$, and 1018.5 $[M+K]^+$ observed for $C_{43}H_{81}NO_{23}$ (979.51).

4.3. Synthesis of the trivalent mannoside head groups

4.3.1. Tris-{2-[(6′-deoxy-1′-*O*-methyl-α-D-mannopyranos-6′-yl)carbamoyl]ethyl}-nitromethane (15). To a mixture of the tris-carboxylic acid core **13** (100 mg, 0.36 mmol), the methyl mannopyranoside **14** (314 mg, 1.29 mmol) and HATU (550 mg, 1.44 mmol) in dry DMF (10 mL), DIPEA (0.50 mL, 2.89 mmol) was added at rt under argon atmosphere. The reaction mixture was heated up to 45 °C for 12 h and then concentrated in vacuo. The resulting crude product was a pale yellowish syrup, which was subjected to purification by gel permeation chromatography on Bio-gel P-2 to finally lead to **15** (265 mg, 0.33 mmol, 92%) as a white lyophilisate (hygroscopic); $R_f = 0.4$ (7:3:1 ^{i}PrOH/H_2O/NH_3, α-naphthol); $[\alpha]_D$ +45 (*c* 1.1 in MeOH), lit.[23] $[\alpha]_D$ +65 (*c* 1.0 in water); ^1H NMR (400 MHz, [d_4]-MeOH): δ 4.62 (d, $J_{1,2} = 1.7$ Hz, 3H, 3H-1), 3.78 (dd, $J_{1,2} = 1.7$, $J_{2,3} = 3.4$ Hz, 3H, 3H-2), 3.65 (dd, $J_{2,3} = 3.4$, $J_{3,4} = 9.0$ Hz, 3H, 3H-3), 3.55–3.46 (m, 12H, 3H-4, 3H-5, 3H-6a, 3H-6b), 3.36 (s, 9H, $3OCH_3$), 2.29–2.20 (m, 12H, $6CH_2$); ^{13}C NMR (100.61 MHz, [d_4]-MeOH): δ 174.4, 173.6 (3CONH), 101.7 (3C-1), 93.3 (C_q-NO_2), 71.2 (3C-4), 71.1 (3C-3), 70.9 (3C-2), 68.3 (3C-5), 54.3 ($3OCH_3$), 40.3 (3C-6), 31.1, 30.0 ($3CH_2CO$), 29.4, 28.0 ($3CH_2CH_2CO$); MALDI-TOFMS: $m/z = 803.4$ $[M+H]^+$, 825.4 $[M+Na]^+$, and 841.4 $[M+K]^+$ observed for $C_{31}H_{54}N_4O_{20}$ (802.33). Anal. Calcd for $C_{31}H_{54}N_4O_{20}$: C, 46.38; H, 6.78; N, 6.98. Found: C, 46.69; H, 6.79; N, 7.11.

4.3.2. Tris-{2-[(6′-deoxy-1′-*O*-methyl-α-D-mannopyranos-6′-yl)carbamoyl]ethyl}-aminomethane (16). A mixture of the cluster mannoside **15** (265 mg, 0.33 mmol), ammonium formate (145 mg, 2.30 mmol), and Pd-catalyst (10% on charcoal, 50 mg) in dry MeOH (5 mL) was hydrogenated at rt under atmospheric pressure. After 6 h, an additional amount of the Pd-catalyst (50 mg) was added and hydrogenation was continued for 24 h. The mixture was filtered through a thin Celite bed and the filtrate was concentrated. The crude product was dissolved in H_2O and lyophilized twice in order to remove excess ammonium formate left in the product mixture. Then it was purified by gel permeation chromatography on Bio-gel P-2 to afford **16** (200 mg, 0.26 mmol, 79%) as a white lyophilisate (hygroscopic); $R_f = 0.3$ (7:3:1 ^{i}PrOH/H_2O/NH_3, α-naphthol); $[\alpha]_D$ +44 (*c* 1.1 in H_2O); ^1H NMR (400 MHz, D_2O): δ 4.61 (d, $J_{1,2} = 1.7$ Hz, 3H, 3H-1), 3.79 (dd, $J_{1,2} = 1.7$, $J_{2,3} = 3.4$ Hz, 3H, 3H-2), 3.62 (dd, $J_{2,3} = 3.4$, $J_{3,4} = 9.1$ Hz, 3H, 3H-3), 3.55–3.45 (m, 9H, 3H-6a, 3H-4, 3H-6b), 3.44–3.34 (m, 3H, 3H-5), 3.33 (s, 9H, $3OCH_3$), 2.26–2.12 (m, 6H, $3CH_2CO$), 1.70–1.51 (m, 6H, $3CH_2CH_2CO$); ^{13}C NMR (100.61 MHz, D_2O): δ 176.5 (3CONH), 101.3 (3C-1), 71.0 (3C-4), 70.8 (3C-3), 70.4 (3C-2), 68.3 (3C-5), 54.7 ($3OCH_3$), 52.7 (C_q–NH_2),

40.3 (3C-6), 35.0 ($3CH_2CH_2CO$), 30.4 ($3CH_2CO$); MALDI-TOFMS: $m/z = 773.3$ $[M+H]^+$, 774.3 $[M+2H]^+$, and 795.3 $[M+Na]^+$ observed for $C_{31}H_{56}N_4O_{18}$ (772.35).

4.3.3. (*tert*-Butyloxycarbonylamido)-triglycine (17). To triglycine (169 mg, 0.89 mmol) dissolved in a mixture of satd $NaHCO_3$ solution/DMF (4:3, 3 mL), di-*tert*-butyl dicarbonate (234 mg, 1.07 mmol) was added at 0 °C and the reaction mixture was stirred at rt for 1 h. Then the solution was neutralized with ice-cold aq HCl solution (5%) at 0 °C. The DMF was removed in vacuo and the crude product so obtained was purified by gel permeation chromatography on Bio-gel P-2 with water as the eluent to afford **17** (245 mg, 0.85 mmol, 96%) as a white lyophilisate (hygroscopic); $R_f = 0.5$ (1:1 MeOH/CH_2Cl_2, UV, α-naphthol); ^1H NMR (300 MHz, D_2O): δ 3.99 (s, 2H, CH_2CO), 3.82 (s, 2H, CH_2CO_2H), 3.79 (s, 2H, CH_2CO), 1.42 (s, 9H, *t*-Bu); ^{13}C NMR (75.46 MHz, D_2O): δ 176.2 (CO_2H), 172.2, 171.7 (2CONH), 158.7 (BocCO), 80.9 ($C(CH)_3$), 44.9, 43.2, 41.8 ($3CH_2$), 28.7, 28.6 ($C(CH)_3$). Anal. Calcd for $C_{11}H_{19}N_3O_6$: C, 45.67; H, 6.62; N, 14.53. Found: C, 45.39; H, 6.79; N, 14.11.

4.3.4. [(*tert*-Butyloxycarbonylamido)-triglycylamido]-tris-{2-[(6′-deoxy-1′-*O*-methyl-α-D-mannopyranos-6′-yl)carbamoyl]ethyl}-methane (18). To a mixture of the cluster **16** (47 mg, 0.06 mmol), *N*-Boc-triglycine **17** (26 mg, 0.09 mmol) and HATU (35 mg, 0.09 mmol) in absolute DMF (2 mL), DIPEA (0.03 mL, 0.17 mmol) were added at rt under argon atmosphere. The reaction mixture was heated at 50 °C for 24 h and then concentrated in vacuo. The resulting crude product was a pale yellowish syrup, which was subjected to purification by gel permeation chromatography on Bio-gel P-2 to afford the title compound **18** (38 mg, 0.04 mmol, 67%) as a white lyophilisate (hygroscopic); $R_f = 0.7$ (7:3:1 ^{i}PrOH/H_2O/NH_3, α-naphthol); $[\alpha]_D$ +32 (*c* 1.01 in H_2O); ^1H NMR (500 MHz, D_2O): δ 4.68 (d, $J_{1,2} = 1.7$ Hz, 3H, 3H-1), 3.93 (s, 2H, CH_2CO), 3.88 (dd, $J_{1,2} = 1.7$, $J_{2,3} = 3.4$ Hz, 3H, 3H-2), 3.80 (s, 2H, CH_2CO), 3.78 (s, 2H, CH_2CO), 3.68 (dd, $J_{2,3} = 3.4$, $J_{3,4} = 9.1$ Hz, 3H, 3H-3), 3.60–3.47 (m, 9H, 3H-4, 3H-6a, 3H-6b), 3.40–3.32 (m, 3H, 3H-5), 3.30 (s, 9H, $3OCH_3$), 2.23–2.11 (m, 6H, $3CH_2CO$), 1.98–1.86 (m, 6H, $3CH_2CH_2CO$), 1.35 (s, 9H, *t*-Bu); ^{13}C NMR (125.75 MHz, D_2O): δ 176.5 (3CONH), 170.4 (3 gly-CONH), 149.1 (BocCO), 101.2 (3C-1), 82.1 (Boc t-C), 71.0 (3C-4), 70.8 (3C-3), 70.3 (3C-2), 68.5 (3C-5), 58.6 ($C(CH)_3$), 55.0 ($3OCH_3$), 43.3, 42.9 ($3NHCH_2CO$), 40.4 (3C-6), 30.5, 30.3 ($3CH_2CH_2CO$, $3CH_2CO$), 28.0 ($C(CH)_3$); MALDI-TOFMS: $m/z = 1066.5$ $[M+Na]^+$ and 1082.5 $[M+K]^+$ observed for $C_{42}H_{73}N_7O_{23}$ (1043.47). Anal. Calcd for $C_{42}H_{73}N_7O_{23}$: C, 48.32; H, 7.05; N, 9.39. Found: C, 48.87; H, 7.01; N, 9.11.

4.3.5. (Triglycylamido)-tris-{2-[(6′-deoxy-1′-*O*-methyl-α-D-mannopyranos-6′-yl)carbamoyl]ethyl}-methane (19). The *N*-Boc-protected cluster **18** (74 mg, 0.07 mmol) was dissolved in a freshly prepared solution of Me_2S/CF_3CO_2H (1:2; 2 mL) at 0 °C and stirred for 3 h. The reaction mixture was concentrated and the last traces of CF_3CO_2H were neutralized with satd NH_3/H_2O solution (1 mL) at 0 °C. After lyophilization the crude product was passed through a filter column of Bio-gel P-2, which afforded the corresponding amine **19** (60 mg, 0.06 mmol, 86%) as a white lyophilisate. $R_f = 0.3$ (7:3:1 ^{i}PrOH/H_2O/NH_3, α-naphthol). MALDI-TOFMS: $m/z = 966.4$ $[M+Na]^+$ and 982.3 $[M+K]^+$ for $C_{37}H_{65}N_7O_{21}$ (calcd 943.42); ESIMS (*m*/*z*): 966.4139; $[M+Na]^+$ (calcd 966.4130).

4.4. Synthesis of nonavalent mannoside clusters

4.4.1. Tris-[3-{(2-[bis-(2-ethoxy)]-carbonylamido)-tris-[2′-[(6″-deoxy-1″-*O*-methyl-α-D-mannopyranos-6″-yl)carbamoyl]ethyl]-methyl}-propyloxy]-nitromethane (20). The triester derivative **6** (30 mg, 41.9 μmol) was dissolved in MeOH/H_2O (1:2; 2 mL) and treated with $LiOH{\cdot}H_2O$ (6.0 mg, 142 μmol) at 0 °C for 12 h. Then the basic reaction mixture was neutralized (pH 6) at 0 °C with HCl (2 N) solution and was freeze-dried to afford the crude triacid core **7** (28 mg, 41.6 μmol, quant.) as a white lyophilisate. The crude **7** (13 mg, 19.3 μmol) was dissolved in dry DMF (3 mL) and the amino-terminated mannoside cluster **16** (60 mg, 77.0 μmol), HATU (26 mg, 69.0 μmol), and DIPEA (30 μL, 173 μmol) were added at rt under argon atmosphere. The reaction mixture was heated at 45 °C for 12 h and then DMF was removed in vacuo. The resulting crude product was a pale yellowish syrup and was subjected to two gel permeation chromatography purifications, on Bio-gel P-2 and P-6, successively. This afforded the title polyethylene ether spaced glycodendron **20** (23 mg, 7.8 μmol, 41%) as a white lyophilisate (hygroscopic); $R_f = 0.1$ (7:3:1 ^{i}PrOH/H_2O/NH_3, α-naphthol). $[\alpha]_D$ +40 (*c* 1.0 in H_2O); ^1H NMR (500 MHz, D_2O): δ 4.7 (d, $J_{1,2} = 1.7$ Hz, 9H, 9H-1), 3.95 (s, 6H, $3OCH_2CO$), 3.9 (dd, $J_{1,2} = 1.6$, $J_{2,3} = 3.3$ Hz, 9H, 9H-2), 3.73 (dd, $J_{2,3} = 3.5$, $J_{3,4} = 9.4$ Hz, 9H, 9H-3), 3.75–3.58 (m, 48H, 9H-5, $J_{6a,6b} = 14.7$ Hz, $15OCH_2$, 9H-6a), 3.54 (dd, $J_{3,4} = 9.5$ Hz, 9H, 9H-4), 3.42 (dd, $J_{5,6b} = 7.5$, $J_{6a,6b} = 14.7$ Hz, 9H, 9H-6b), 3.33 (s, 27H, $9OCH_3$), 2.30–2.15 (m, 18H, $9CH_2$), 2.05–1.90 (m, 24H, $12CH_2$), 1.55–1.35 (m, 6H, $3CH_2$); ^{13}C NMR (125.75 MHz, D_2O): δ 175.9, 171.3 (12CONH), 100.9 (9C-1), 95.1 (NO_2–C_q), 70.6 (9C-5), 70.5 ($3OCH_2$), 70.4 (9C-3, $3OCH_2$), 70.3 ($3OCH_2$), 69.9 (9C-2, $3OCH_2$), 69.7, 69.5, 69.2, 69.1 ($6OCH_2$), 68.1 (9C-4), 60.6 (3NH–C_q), 54.6 ($9OCH_3$), 40.1 (9C-6), 31.6 ($3CH_2$), 30.0, 29.9 ($18CH_2$), 23.1 ($3CH_2$); MALDI-TOFMS: $m/z = 2959.3$ $[M+Na]^+$, 2975.3 $[M+K]^+$

observed for $C_{121}H_{213}N_{13}O_{68}$ (2936.36); ESIMS (*m*/*z*): 2937.3649 $[M+H]^+$ (calcd 2937.3687).

4.4.2. Tris-[3-{(2-[tetrakis-(2-ethoxy)]-carbonylamido)-tris-[2′-[(6″-deoxy-1″-*O*-methyl-α-D-mannopyranos-6″-yl)-carbamoyl]ethyl]-methyl}-propyloxy]-nitromethane (21). The triester derivative **11** (60 mg, 64.0 μmol) was dissolved in MeOH/H_2O (1:2; 2 mL) and treated with LiOH·H_2O (14 mg, 330 μmol) at 0 °C for 12 h. Then the basic reaction mixture was neutralized (pH 6) at 0 °C with HCl (2 N) solution and was freeze-dried to afford the crude triacid **12** (60 mg, 64.0 μmol, quant.) as a white lyophilisate. The crude derivative **12** (19 mg, 20.0 μmol) was dissolved in dry DMF (3 mL) and the amino-terminated mannoside cluster **16** (62 mg, 80.0 μmol), HATU (27 mg, 72.0 μmol), and DIPEA (0.03 mL, 180 μmol) were added at rt under argon atmosphere. The reaction mixture was heated at 45–50 °C for 12 h and then DMF was removed in vacuo. The resulting pale syrup was subjected to two successive gel permeation chromatography steps on Bio-gel P-2, and P-6 to get the title glycodendron **21** (24 mg, 7.5 μmol, 38%) as a white lyophilisate (hygroscopic); $R_f = 0.3$ (7:3:1 ^{i}PrOH/H_2O/NH_3, α-naphthol); $[\alpha]_D$ +40 (*c* 0.8 in H_2O); ^{1}H NMR (500 MHz, D_2O): δ 4.58 (d, $J_{1,2} = 1.8$ Hz, 9H, 9H-1), 3.86 (s, 6H, 3OCH_2CO), 3.76 (dd, $J_{1,2} = 1.7$, $J_{2,3} = 3.5$ Hz, 9H, 9H-2), 3.56 (dd, $J_{2,3} = 3.5$, $J_{3,4} = 9.3$ Hz, 9H, 9H-3), 3.60–3.35 (m, 54H, 27OCH_2), 3.56–3.43 (m, 18H, 9H-5, 9H-6a), 3.39 (dd, $J_{3,4} = 9.3$ Hz, 9H, 9H-4), 3.27 (dd, 9H, 9H-6b), 3.22 (s, 27H, 9OCH_3), 2.17–2.08 (m, 18H, 9CH_2), 1.92–1.83 (m, 24H, 12CH_2), 1.28–1.26 (m, 6H, 3CH_2); ^{13}C NMR (125.75 MHz, D_2O): δ 175.3, 170.7 (12CONH), 100.3 (9C-1), 94.6 (NO_2–Cq), 70.0 (9C-5), 69.8 (9C-3), 69.8 (3OCH_2, 9C-2), 69.7, 69.3, 69.2, 69.1, 69.0, 68.9, 68.6 (27OCH_2), 67.5 (9C-4), 60.6 (3NH–Cq), 54.0 (9OCH_3), 39.5 (9C-6), 31.0, 29.4, 29.3 (21CH_2), 22.5 (3CH_2); MALDI-TOFMS: $m/z = 3184.7$ $[M-16]^+$, 3200.6 $[M]^+$, 3223.7 $[M+Na]^+$, 3239.6 $[M+K]^+$ observed for $C_{133}H_{237}N_{13}O_{74}$ (3200.51); ESMS (*m*/*z*): 3201.5289 $[M+H]^+$ (calcd 3201.5260).

4.4.3. Tris-{2-[carbonylamido-(triglycylamido)-tris-{2′-[(6″-deoxy-1″-*O*-methyl-α-D-mannopyranos-6″-yl)carbamoyl]ethyl}-methyl]-ethyl}-nitromethane (22). Peptide coupling between 4-(2-carbethoxy)-4-nitro-heptan-1,7-dicarboxylicacid **13** (3.2 mg, 11.0 μmol) and the spaced cluster **19** (44 mg, 46.0 μmol) was performed using HATU (17 mg, 40.0 μmol) and DIPEA (0.02 mL, 120 μmol) in dry DMF (3 mL). The reaction mixture was stirred under argon atmosphere at 45–50 °C for 12 h and then concentrated in vacuo. The resulting pale syrup was subjected to two successive gel permeation chromatography steps, first on Bio-gel P-2, then on Bio-gel P-6 to get the triglycine-spaced glycodendron **22** (10 mg, 3.2 μmol, 29%) as a white lyophilisate (hygroscopic); $R_f = 0.1$ (7:3:1 ^{i}PrOH/H_2O/NH_3, α-naphthol); $[\alpha]_D$ +35 (*c* 1.0 in H_2O); ^{1}H NMR (500 MHz, D_2O): δ 4.68 (br s, 9H, 9H-1), 3.89 (s, 6H, 3NHCH_2CO), 3.87 (s, 6H, 3NHCH_2CO), 3.83 (dd, $J_{1,2} = 1.7$, $J_{2,3} = 3.5$ Hz, 9H, 9H-2), 3.76 (s, 6H, 3NHCH_2CO), 3.64 (dd, $J_{2,3} = 3.5$, $J_{3,4} = 9.4$ Hz, 9H, 9H-3), 3.55–3.49 (m, 18H, 9H-6a, 3H-6b), 3.46 (dd, $J_{3,4} = 9.5$ Hz, 9H, 9H-4), 3.37–3.31 (m, 9H, 9H-5), 3.28 (s, 27H, 9OCH_3), 2.3–2.19 (m, 12H, 6CH_2), 2.18–2.14 (m, 18H, 9CH_2), 1.92–1.86 (m, 18H, 9CH_2); ^{13}C NMR (125.75 MHz, D_2O): δ 176.5, 175.5 (9CONH), 172.6, 172.4, 170.4 (12gly-CONH), 101.2 (9C-1), 93.6 (NO_2–C_q), 71.0 (9C-4), 70.8 (9C-3), 70.3 (9C-2), 68.4 (9C-5), 58.7 (3NH–C_q), 55.1 (9OCH_3), 43.3, 43.0, 42.9 (9NH*C*H_2CO), 40.5 (9C-6), 30.7, 30.4, 30.2, 30.1 (24CH_2); MALDI-TOFMS: $m/z = 3076.1$ $[M+Na]^+$ observed for $C_{121}H_{204}N_{22}O_{68}$ (3053.3); ESIMS (*m*/*z*): 3054.3288 $[M+H]^+$ (calcd 3054.3259).

4.4.4. 1,3,5-{Tris-[(triglycylamido)-tris-(2′-[(6″-deoxy-1″-*O*-methyl-α-D-mannopyranos-6″-yl)carbamoyl]ethyl)-methyl]} benzene-triamide (24). Trimesic acid (**23**) (2.2 mg, 10.0 μmol), the mannoside cluster **19** (41 mg, 40.0 μmol), HATU (15 mg, 40.0 μmol), and DIPEA (0.02 mL, 110 μmol) were dissolved in dry DMF (3 mL) under argon atmosphere. The reaction mixture was stirred at 45–50 °C for 12 h and then concentrated in vacuo. The resulting syrup was subjected to two successive gel permeation chromatography steps, first on Bio-gel P-2, then on Bio-gel P-6 to get the title triglycine spaced glycodendrimer **24** (10 mg, 3.3 μmol, 33%) as a white lyophilisate (hygroscopic); $R_f = 0.1$ (7:3:1 ^{i}PrOH/H_2O/NH_3, α-naphthol); $[\alpha]_D$ +33 (*c* 0.6 in H_2O); ^{1}H NMR (500 MHz, D_2O): δ 8.50 (s, 3H, 3aryl-H), 4.70 (br s, 9H, 9H-1), 4.22 (s, 6H, 3NHCH_2CO), 4.01 (s, 6H, 3NHCH_2CO), 3.90 (dd, $J_{1,2} = 1.7$, $J_{2,3} = 3.5$ Hz, 9H, 9H-2), 3.86 (s, 6H, 3NHCH_2CO), 3.71 (dd, $J_{2,3} = 3.5$, $J_{3,4} = 9.3$ Hz, 9H, 9H-3), 3.61–3.49 (m, 27H, 9H-4, 9H-6a, 3H-6b), 3.40–3.36 (m, 9H, 9H-5), 3.35 (s, 27H, 9OCH_3), 2.22–2.15 (m, 18H, 9CH_2), 1.98–1.90 (m, 18H, 9CH_2); ^{13}C NMR (125.75 MHz, D_2O): δ 176.4 (9CONH), 172.5, 172.4, 170.5, 169.0 (12gly-CONH), 134.6 (3aryl-C_q), 130.2 (3aryl-CH), 101.2 (9C-1), 71.0 (9C-4), 70.7 (9C-3), 70.3 (9C-2), 68.5 (9C-5), 58.7 (3NH–C_q), 55.0 (9OCH_3), 43.7, 43.4, 43.1 (9NHCH_2CO), 40.4 (9C-6), 30.4, 30.2 (18CH_2); ESIMS (*m*/*z*): 2496.0524 $[M+Na]^+$ (calcd 2473.0616 for $C_{102}H_{168}N_{12}O_{57}$).

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