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One-pot preparation of a series of glycoconjugates with predetermined antigen–carrier ratio from oligosaccharides that mimic the O-PS of *Vibrio cholerae* O:1, serotype Ogawa

Rina Saksena, Xingquan Ma, Pavol Kováč*

Laboratory of Medicinal Chemistry (LMC), NIDDK, National Institutes of Health, Building 8, Rm B1A24, Bethesda, MD 20892-0815, USA

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Abstract

Di-through the pentasaccharide that mimic the upstream terminus of the O-specific polysaccharide of *Vibrio cholerae* O:1, serotype Ogawa were synthesized in the form of 5-methoxycarbonylpentyl glycosides and linked to BSA using squaric acid diester chemistry. The conjugation reactions were monitored by surface-enhanced laser-desorption/ionization-time-of-flight mass spectrometry (SELDI-TOF MS), which allowed conducting the conjugation of the synthetic oligosaccharides in a controlled way and termination of the reaction when the desired molar hapten–BSA ratio had been reached. This made it possible to prepare, from one hapten in a one-pot reaction, a series of neoglycoconjugates having different, predetermined carbohydrate–carrier ratios. The accuracy of molecular mass determination in SELDI-TOF MS analysis could be increased by using the carrier protein as the internal standard.

Keywords: *Vibrio cholerae* O:1; Synthetic oligosaccharides; Vaccine; Neoglycoconjugate immunogens; SELDI-TOF

1. Introduction

The O-specific polysaccharides (O-PSs) of the two main strains of *Vibrio cholerae*, Ogawa and Inaba, consist of a chain of α-(1→2)-linked 4-amino-4,6-dideoxy-D-mannopyranose (D-perosamine), the amino group of which is acylated with 3-deoxy-L-*glycero*-tetronic acid. Only the Ogawa strain has the O-2 of its upstream, terminal end moiety of perosamine methylated (Fig. 1).[1,2] Continued occurrence of cholera in the third, as well as in the developed world, augments the need for an effective and safe vaccine for this serious enteric disease. In contrast to recent approaches utilizing molecular biology,[3] bacteriology,[4] or bacterial polysaccharides,[5,6] our efforts, as well as those of others,[7] have been focused towards developing a conjugate vaccine for cholera from synthetic fragments of the O-PS. We have previously reported[8] that neoglycoconjugates from the hexasaccharide that mimics the upstream terminus of the O-PS of *Vibrio cholerae* O:1, serotype Ogawa linked to bovine serum albumin (BSA) showed vibriocidal activity and protective capacity in neonatal mice and, thus, potential as vaccines for cholera. In order to establish the effect of the size of the antigenic oligosaccharide upon immunogenicity and protective capacity, we have now synthesized a large series of similar neoglycoconjugates from mono-through the pentasaccharide fragments of the O-PS, and have characterized them by the carbohydrate/BSA ratio (Scheme 1). The serological evaluation of the materials reported herein will be communicated elsewhere in due time.

* Corresponding author. Tel.: +1-301-4963569; fax: +1-301-4020589.
E-mail address: kpn@helix.nih.gov (P. Kováč).

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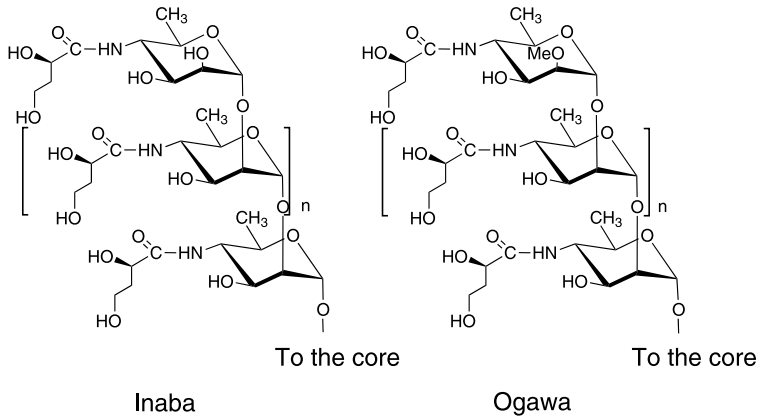
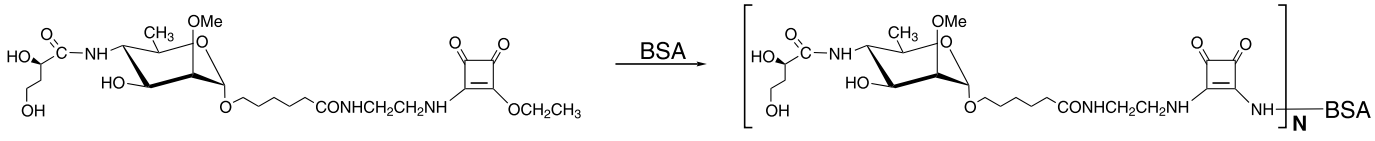


Fig. 1. Structure of the O-PS of *V. cholerae* O1, serotypes Inaba and Ogawa.

2. Results and discussion

The O-PSs of *V. cholerae* O:1, serotypes Inaba and Ogawa are the protective antigens for the disease caused by these pathogens.[9,10] Our previous solution[11] and X-ray crystallographic[12] studies identified the upstream terminus of the O-PS of *V. cholerae* O:1, serotype Ogawa as the dominant determinant epitope. That structural motif was included in the neoglycoconjugates prepared from the synthetic hexasaccharide,[13] which showed potential as vaccines for cholera.[8] The linker equipped monosaccharide hapten **1** required during this work was prepared as described previously.[14] The requisite di-through the pentasaccharides **7**–**10** (Scheme 2) were obtained from the intermediate 2-*O*H derivatives (**3**–**5**), which were obtained during syntheses of substances in the Inaba series (**3**–**6**),[15] namely by methylation or glycosylation using the 2-O-methylated glycosyl donor **28**. Thus, methylation[16,17] of alcohols **3**–**5** with MeI and Ag_2O gave the fully protected substances **7**–**9**, which were subjected to hydrogenolysis to afford the deprotected methyl esters **11**–**13**. We also explored preparation of the desired, fully protected oligosaccharides **7**–**10** through the alternative route involving glycosylation of **3**–**5** with **28** because the methylation, especially of higher oligosaccharides, was impractically slow, and was also accompanied by a side reaction. Therefore, methylation of **6** was not pursued, and the pentasaccharide **10** was only obtained by glycosylation of **5** with **28**.

Previously,[18] when a hexasaccharide similar to **3**–**6** but lacking the tetronamido side chain was methylated under the above conditions the reaction was a clean, one product reaction. On the contrary, methylation of each of the oligosaccharides **3**–**5** at the above conditions was accompanied by formation of variable amounts of a by-product. Based on the NMR and high-resolution mass spectral data, the compound formed during methylation of the disaccharide **3** was identified as the methoxycarbonyl derivative **29**. The molecular formula, $C_{57}H_{70}N_2O_{17}$, was deduced from the HRMS data. Compared to the ^{1}H NMR spectrum of the desired product **7**, the spectrum of **29** lacked the signal for the ethereal Me group at δ 3.28 but showed an additional singlet (OCH_3, δ 3.74), and the signal of H-2^{II} was shifted downfield (δ 5.30), indicating the presence of an electron-withdrawing (CH_3OCOO) group at C-2. The ^{13}C NMR spectrum of **29** showed the signal of the OCH_3 group at δ 51.46, and the signal for C-2^{II} appeared at δ 70.90, showing that *O*-2 was not alkylated (c.f. the chemical shift for *C*-2 in the spectrum of the 2-*O*-methyl derivative **7**, δ 75.72). Taking into consideration the foregoing structurally significant spectral data, the structure **29** was further substantiated when treatment of **29** with NaOMe in methanol regenerated the starting alcohol **3**. The same product **29** was formed when either freshly made or commercial Ag_2O was used. The details concerning the formation of **29** during methylation of **3** or, presumably, analogous products when oligosaccharides **4** and **5** were methylated in the



1

N	
2a	5.3
2b	9.7
2c	15.2

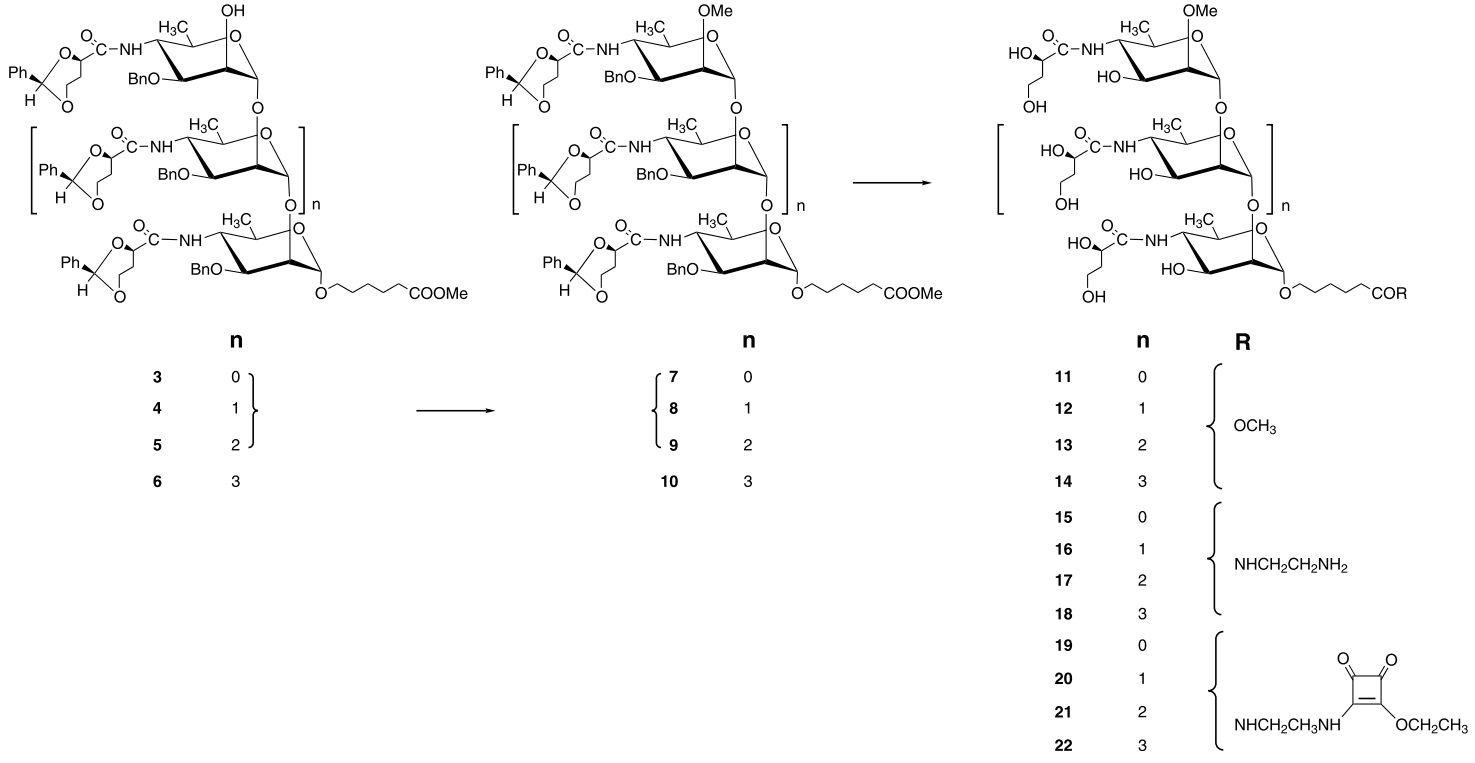
Scheme 1.

same way, were not investigated further. However, it appears that, among other factors, a carbonate must have played role in this side reaction. A plausible source of a carbonate could be a small amount of $BaCO_3$ present in the Ag_2O used. The former was likely to have been present in $Ba(OH)_2$, which is the common reagent used in preparation[16] of Ag_2O. Since $BaCO_3$ is insoluble in water, once present in the Ag_2O formed from $AgNO_3$ and $Ba(OH)_2$, it is virtually impossible to wash it out. Thus, $BaCO_3$ could have been carried over to the product Ag_2O, and could conceivably contribute to the occurrence of the side reaction. It is noteworthy that the formation of side products was not observed when the same batch of reagents were used in the conversion **27**→**28** or in the methylation of methyl 3-*O*-benzyl-4,6-benzylidene-β-D-glucopyranoside.

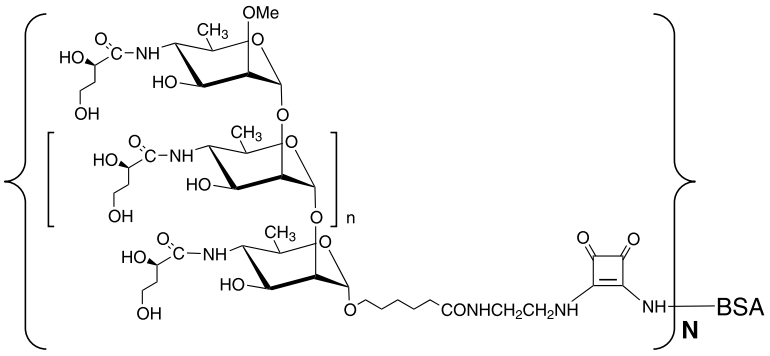
To form substances amenable to conjugation by the squaric acid chemistry, each of the methyl esters **11**–**14** was subjected to aminolysis with excess of ethylenediamine, and the amines formed (**15**–**18**, respectively) were subsequently converted to the squaric acid monoesters **19**–**22**. Purification of amines and squaric acid derivatives was most conveniently done by solid phase extraction and, if necessary, followed by preparative TLC on normal phase silica gel. The NMR spectra showed remarkable purity of materials thus obtained. The same technique and mass spectrometry fully confirmed the expected structures (c.f. Section 3 for the *m*/*z* values and for characteristic resonances present in the ^1H and ^{13}C NMR spectra of **15**–**18** and **19**–**22**).

For conjugation, we chose the method originally described by Tietze, which is based on the selective reaction of amines with squaric acid diesters.[19,20] The method is simple, it requires inexpensive, commercially available reagents, and it has gained popularity in synthetic vaccine development because it allows preparation of neoglycoconjugates with a wide range of hapten–carrier ratios.[14,18,21–24] Further advantages of the method are that its use does not result in cross-linked, poorly defined, lattice type products, and that a some of the excess hapten used at the onset of the reaction can be recovered.[25] We have done some fundamental work on conjugation of synthetic oligosaccharides using squaric acid chemistry[14,18,26,27] and, with the positive results of our own and of others at hand,[8,28,29] we are confident that this method of conjugation will be useful in synthetic vaccine preparation. With only minor modifications, conjugations here described were performed applying the two stage protocol involving purification of the squaric acid monoesters,[23] (c.f. for a simplified procedure[24]) as it is more likely to consistently afford well defined products, clearly a requirement when such materials would be used as vaccines.

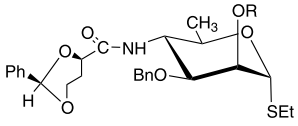
We targeted our carbohydrate–BSA constructs (**2a**–**c** and **23a**–**26c**) to have a hapten–BSA ratio of ∼5, ∼10 and ∼15. To maintain an acceptable reaction rate, the



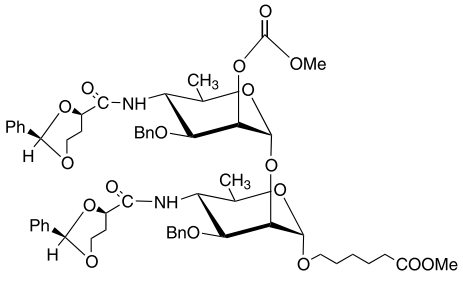
Scheme 2.



	n	N
23a	0	4.7
23b	0	9.7
23c	0	14.5
24a	1	4.9
24b	1	9.8
24c	1	13.9
25a	2	5.9
25b	2	9.0
25c	2	13.9
26a	3	5.0
26b	3	10.5
26c	3	13.9



	R
27	OH
28	Me



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molar hapten–BSA ratio at the onset of the conjugation reaction was 75, which was fivefold excess based on the highest targeted loading. All reactions were carried out under the same conditions, including the 15 mmolar concentration of the hapten.[14] The targeted hapten–BSA ratios and those observed in the final products, and the reaction times required to reach the final loading are in Table 1. Fig. 2 shows the overall course of the conjugations. It can be seen that there is virtually no difference between the rate of conjugation of the mono- and the disaccharide. With the higher oligosaccharides, the reaction rate decreases markedly at the later stages of the reaction, when these larger molecules have to penetrate deeper into the three-dimensional structure of the protein, to reach the less accessible amino groups present in the protein. We cannot explain the anomalous, apparently somewhat higher rate of the conjugation of the tetrasaccharide, compared to that of the trisaccharide.

This laboratory was the first to show the utility of surface-enhanced laser desorption/ionization time-of-flight mass spectrometry (SELDI-TOF MS) in combination with the ProteinChip® System for monitoring the conjugation of synthetic oligosaccharides to pro-

teins.[25,26] The ability to monitor conjugation reactions and obtain virtually real time information about the increasing molecular mass of products being formed constitutes a new milestone in the preparation of neoglycoconjugates. As we show below, a series of such materials can be efficiently prepared from one hapten in a one-pot reaction. According to the protocol described in the Experimental, when the conjugation reaction is monitored by SELDI-TOFMS, a portion of the reaction mixture can be withdrawn and processed when the desired carbohydrate–protein ratio is reached. The rest of the material is allowed to react until the conjugate shows the next desired, predetermined molecular weight. Following this protocol, we have been able to prepare three different neoglycoconjugates with very close to predetermined hapten–carrier ratio from each of the haptens **1** and **19**–**22**. The use of the carrier protein as an internal standard (see Section 3) increased the accuracy and reliability of molecular mass determination by SELDI-TOFMS. This was particularly helpful with conjugates **2a**–**2c** made from the monosaccharide hapten. With these materials, the molecular mass deviation from the actual value allowed by the manufacturer of the instrument (0.3%) can amount to a considerable fraction of one monosaccharide hapten. The extra operation involved in the protocol is simple, and the method can be also useful in routine matrix-assisted LDI-TOFMS.

Table 1
The targeted and observed hapten–BSA ratios in the product conjugates, and the respective reaction time [a]

Hapten/Product	Loading (hapten/BSA)		Time required (h)
	Targeted	Observed	
1/2a	5	5.3	1.25
1/2b	10	9.7	3.75
1/2c	15	15.2	11.0
19/23a	5	4.7	1.0
19/23b	10	9.7	4.0
19/23c	15	14.5	11.5
20/24a	5	4.9	3.0
20/24b	10	9.8	7.0
20/24c	15	13.9	16.75
21/25a	5	5.9	3.0
21/25b	10	9.0	5.75
21/25c	15	13.9	15.0
22/26a	5	5.0	3.0
22/26b	10	10.5	9.0
22/26c	15	13.9	21.5

[a] All reaction were carried out at ambient temperature (23–26 °C) at the initial molar hapten–BSA ratio of 75, and at the hapten concentration of 15 mmol.

3. Experimental

3.1. General methods

Instruments and laboratory techniques used were the same as those described previously.[25,30] Unless stated otherwise, optical rotations were measured at ambient temperature for solutions in chloroform ($c \sim 1$), with a Perkin–Elmer automatic polarimeter, Model 341. All reactions were monitored by thin-layer chromatography (TLC) on Silica Gel 60 coated glass slides (Whatman or Analtech). Preparative TLC was performed using Silica Gel GF-coated Analtech Uniplates (1500 μm). Column chromatography was performed by gradient elution from columns of silica gel, using PurChrom 35G High Performance Rapid Chromatography System (RT Scientific, Inc.). Solvent mixtures less polar than those used for TLC were used at the onset of development. NMR spectra were measured at 300 (^{1}H) and 75 MHz (^{13}C), with a Varian Mercury spectrometer. Solid Phase Extraction was performed using Strata SPE Tubes (Phenomenex, Inc.). Assignments of NMR signals were made by first-order analysis of the spectra, and by comparison with spectra of related substances reported previously from this laboratory[13,30–33] or elsewhere.[34] When the latter approach was used to aid in the ^{13}C NMR signal-nuclei assignments, advantage was taken of variations of line intensity expected for oligosaccharides belonging to the same homologous series.[35,36] Thus, spectra showed close similarity of chemical shifts of equivalent carbon atoms of the internal residues, and an increase in the relative intensity of these signals with the increasing number of N-acylated D-perosamine residues in the molecule. When feasible, the assignments were supported by homonuclear decoupling experiments or homonuclear (^{1}H{^{1}H}COSY) and heteronuclear (^{13}C{^{1}H}HETCOR) 2-dimensional correlation spectroscopy, run with the software supplied with the spectrometers. When reporting assignments of NMR signals of oligosaccharides, sugar residues in oligosaccharides are serially numbered, beginning with the one bearing the aglycon, and are identified by a Roman numeral superscript in listings of signal assignments. Nuclei-assignments without a superscript notation indicate that those signals have not been individually assigned. Thus, for example, in a spectrum of a pentasaccharide, a resonance denoted H-3 could be that of H-3 of either sugar residue. When reporting NMR data, the nuclei belonging to the 3-deoxy-L-*glycero*-tetronic acid side chain are referred to as primed (′), and those of the spacer aglycon as double primed (″). Some signals on the ^{13}C NMR spectra of squaric acid derivatives appeared as doublets, the splitting of these signals being due to the vinylogous amide group, characteristic of squaric acid amide esters.[19] Surface-

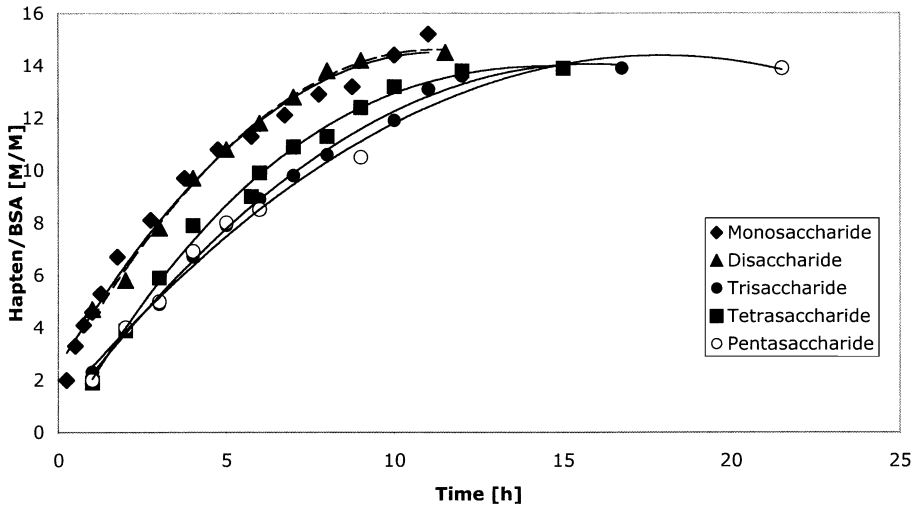

Fig. 2. Rate of conjugation for the mono- through the pentasaccharide. For reaction conditions, see Table 1.

enhanced laser desorption/ionization time-of-flight mass spectrometry (SELDI-TOF MS) was done using PBS-II Mass Reader® (Ciphergen Biosystems, Inc), calibrated using Ciphergen's All in One Protein Standards, and H4 or NP20 Protein Chip Arrays®. The accuracy of molecular mass determination was increased by using the carrier protein (BSA, *m*/*z* 66,430 Da) as an internal standard. This eliminated errors originating from spot-to-spot variations in the SELDI-TOFMS, and made the fine calibration of the instrument virtually unnecessary when used for monitoring the conjugations or molecular mass determination of final products here described. Attempts have been made to obtain correct analytical data for all new compounds. However, some compounds tenaciously retained traces of solvents, despite exhaustive drying, and analytical figures for carbon could not be obtain within $\pm 0.3\%$. Structures of these compounds follow unequivocally from the mode of synthesis and *m*/*z* values found in their low- and high-resolution mass spectra, and TLC and NMR spectroscopy verified their purity. BSA (Fraction V, Prod. No. A-4503) was purchased from Sigma Chemical Company, and purified as described by Chen.[37] Ethylenediamine was freshly distilled, bp 115–116 °C. Palladium-on-charcoal catalyst (5%, ESCAT 103) was a product of Engelhard Industries. Silver oxide was purchased from Fluka Chemical Company, or prepared as described.[16]

3.2. General procedure for preparation of amines 15–18 from esters 11–14

A solution of the ester (0.1 mmol) in ethylenediamine (20 mmol) was heated at 60–70° overnight, when TLC (1.3:1:0.1 CH_2Cl_2–MeOH–25% NH_4OH) showed that the reaction was complete. After concentration and evaporation of water from the residue ($\times 3$), the solution of the crude product in water (~ 2 mL) was applied on a SPE column (2–10 g sorbent mass, as required), which had been conditioned by washing with MeOH (30–80 mL), followed by water (50–100 mL). The elution was effected with water (20–50 mL), followed by a gradient of water (25 mL) → 50% aq MeOH (25 mL). Fractions of 2 mL were collected, analyzed by TLC, and those containing the desired material were concentrated to a small volume. Filtration through a syringe filter (0.45 μm porosity) and freeze-drying, gave products as white solids.

3.3. General procedure for preparation of squaric acid monoesters 19–22

Diethyl squarate (0.015 mmol) was added to a soln of each of the above amines **15**–**18** (0.01 mmol) in a potassium phosphate buffer pH 7.0 (3 mL), and the mixture was kept at room temperature overnight, when TLC (1:1 CH_2Cl_2–MeOH) showed that all the amine had been consumed and that a less polar, UV positive product was formed. Without any work-up, the mixture was subjected to SPE, using a column (sorbent mass, 5 g), which had been conditioned by washing with MeOH (40 mL), followed by water (60 mL). The elution was effected with water (20 mL), followed by a gradient of water (25 mL) → 50% aq MeOH (25 mL). Fractions of 2 mL were collected and analyzed by TLC. Fractions containing the desired material were concentrated and purified by preparative TLC, if necessary. An aq soln of the pure material, thus obtained, was filtered through a syringe filter (0.45 μm porosity) and freeze-dried, to give white or light-yellow solids.

3.4. General procedure for the preparation of neoglycoconjugates 2a–2c, 23a–26c

BSA (20 mg, 0.3 μmol) was suspended in a borate buffer, pH 9.0 (400 μL) and homogenized with the aid of a vortexer. The respective haptenic monoamide (0.0226 mmol) was transferred from its weighing container, using 1.1 mL of phosphate buffer pH 9.0 divided into three portions (400, 400 and 300 μL), into the above fine suspension of BSA. The clear soln thus formed was stirred at room temperature, while the progression of the conjugation was periodically monitored by SELDI-TOF MS, following the protocol recommended by Ciphergen for NP20 ProteinChips®. For monitoring the conjugation using BSA as the internal standard, a sample (1 μL) was withdrawn and mixed with pH 7 phosphate buffer (9 μL), giving soln A. A 1 μL sample of a stock soln of BSA (20 mg/1.5 mL) was diluted with 9 μL of water, yielding solution B (internal standard). A portion of A (2 μL) was mixed with B (1 μL) and 1μL of the resulting soln was applied on the ProteinChip®. The chip was air-dried, washed with water (5 μL, twice), with drying in between the washes and, finally, a soln (0.5 μL, twice) of the energy-absorbing molecule (satd solution of sinapinic acid in 1% TFA) was applied. The chip was air-dried, followed by reading the molecular masses. Two peaks corresponding to single-charged molecules were observed. The average hapten/BSA ratio was calculated from the difference between the molecular masses shown for BSA and the glycoconjugate formed. When the required hapten/BSA ratio was reached, 500 μL of the soln was withdrawn, diluted with phosphate buffer pH 7.0 (3 mL), and subjected to ultra filtration using the Amicon cell equipped with PM-10 membrane (Millipore Copporation, Bedford, MA). The retained material was freeze-dried, to give conjugates in 80–95% yields. The molecular mass of the final products was verified by SELDI-TOFMS.

3.5. 5-Methoxycarbonylpentyl 3-*O*-benzyl-4-(2,4-*O*-benzylidene-3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-2-*O*-methyl-α-D-mannopyranosyl-(1→2)-3-*O*-benzyl-4-(2,4-*O*-benzylidene-3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-α-D-mannopyranoside (7)

(a) A solution of the disaccharide **3**[15] (58 mg, 0.058 mmol) in MeI (2 mL) was treated with Ag_2O (137 mg, ~10 equiv), and the mixture was stirred at room temperature with exclusion of direct light overnight, when TLC (2:1 toluene–acetone) showed that nearly no starting material was present. After filtration, the solids were washed with CH_2Cl_2, the combined filtrate was concentrated, and the residue was chromatographed (5:1→2:1 toluene–acetone), to give first 5-methoxycarbonylpentyl 3-*O*-benzyl-4-(2,4-*O*-benzylidene-3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-2-*O*-methoxycarbonyl-α-D-mannopyranosyl-(1→2)-3-*O*-benzyl-4-(2,4-*O*-benzylidene-3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-α-D-mannopyranoside (**29**, 13 mg, 21%). ^{1}H NMR ($CDCl_3$): δ 6.37, 6.22 (2 d, 1 H each, $J_{4,NH}$ 9.4 Hz, 2 NH), 5.56, 5.54 (2 s, 1 H each, 2 C*H*Ph), 5.30 (broad t, 1 H, H-2^{II}), 4.99 (d, 1 H, $J_{1,2}$ 1.9 Hz, H-1^{II}), 4.66 (d, 1 H, $J_{1,2}$ 1.7 Hz, H-1^{I}), 4.73–4.47 (4 d, 1 H each, ^2J 11.8 Hz, 2 CH_2Ph), 4.38–4.21 (m, 4 H, H-2′^{I,II},4′a^{I,II}), 4.12–3.88 (m, 5 H, H-2^{I},4^{I,II},4′b^{I,II}), 3.86–3.71 (m, 7 H, H-3^{I,II},5^{I,II}, incl. s, 3.74, $OCOOCH_3$), 3.62–3.54 (m, 4 H, H-1″a, incl. s, 3.60 $COOCH_3$), 3.32, 3.29 (2 t, 1 H, *J* 6.2 Hz, H-1″b), 2.77 (t, 2 H, *J* 7.4 Hz, H-5″), 2.10–1.83 (2 m, 2 H, each, H-3′^{I,II}), 1.66–1.50 (m, 4 H, H-4″,2″ in that order), 1.39–1.28 (m, 2 H, H-3″), 1.19, 1.17 (2 d, partially overlapped, 6 H, $J_{5,6}$ 6.0 Hz, H-6^{I,II}); ^{13}C NMR ($CDCl_3$): δ 101.35, 101.19 (2 *C*HPh), 99.45 (C-1^{II}), 98.85 (C-1^{I}), 76.58 (2 C, C-2′^{I,II}), 75.69 (C-3^{I}), 74.27 (C-2^{I}), 73.29 (C-3^{II}), 71.98, 71.12 (2 *C*H$_2$Ph), 70.90 (C-2^{II}), 68.42, 67.42 (C-5^{I,II}), 67.34 (3 C, C-4′^{I,II},1″), 54.84 ($OCOOCH_3$), 52.62, 51.67 (C-4^{I,II}), 51.46 ($COOCH_3$), 33.89 (C-5″), 28.86 (C-2″), 28.62 (C-3′), 25.26 (C-3″), 24.56 (C-4″), 18.00, 17.95 (C-6^{I,II}). FABMS: *m*/*z* 1055.41 ([M+1]$^+$), 1077.39 ([M+Na]$^+$); HRMS: *m*/*z* 1187.3727. $C_{57}H_{70}CsN_2O_{17}$ requires 1187.3661.

Eluted next was the desired methyl ether **7** (41 mg, 70%), $[\alpha]_D$ −15.1° (*c* 0.5). ^{1}H NMR ($CDCl_3$): δ 6.40, 6.22 (2 d, 1 H each, $J_{4,NH}$ 9.8 Hz, 2 NH), 5.57, 5.54 (2 s, 1 H each, 2 C*H*Ph), 5.01 (broad doublet, 1 H, H-1^{II}), 4.71–4.50 (4 d, partially overlapped, 4 CH_2Ph), 4.66 (d, partially overlapped, $J_{1,2}$ ~1.7 Hz, H-1^{I}), 4.42–4.29 (m, 4 H, H-2′^{I,II},4′a^{I,II}), 4.18–4.92 (m, 5 H, H-2^{I},4^{I,II},4′b^{I,II}), 3.80–3.70 (m, 5 H, H-2^{II},3^{I,II},5^{I,II}), 3.63–3.55 (m, 4 H, H-1″, incl s at 3.60, $COOCH_3$), 3.36 (m, 1 H, H-1″b), 3.28 (s, 3 H, OCH_3-2), 2.28 (t, 2 H, *J* 7.4 Hz, H-5″), 2.11–1.82 (m, 4 H, H-3′a,b^{I,II}), 1,67–1.51 (m, 4 H, H-2″a,b^{I,II},4″a,b^{I,II}), 1.38 (m, 2 H, H-3″a,b^{I,II}), 1.19 (m, 6 H, $J_{5,6}$ 6.3 Hz, H-6^{I,II}). ^{13}C NMR ($CDCl_3$): δ 101.29, 101.22 (2 *C*HPh), 99.58 (C-1^{II}), 99.06 (C-1^{II}), 76.50 (2 C, C-2′^{I,II}), 76.47, 75.38 (C-3^{I,II}), 75.72 (C-2^{II}), 73.34 (C-2^{I}), 72.33, 71.06 (*C*H$_2$Ph), 68.55, 67.52 (C-5^{I,II}), 67.33 (C-1″), 67.27 (C-4′^{I,II}), 59.13 (OCH_3-2), 52.44, 51.81 (C-4^{I,II}), 33.86 (C-5″), 28.85 C-2″), 28.58 (C-3′), 25.55 (C-3″), 24.52 (C-4″), 18.04, 17.95 (C-6^{I,II}). FABMS: *m*/*z* 1011.42 ([M+1]$^+$), 1033.40 ([M+Na]$^+$). HRMS: *m*/*z* 1143.3843. $C_{56}H_{70}CsN_2O_{15}$ requires 1143.3831. Anal. Calcd for $C_{56}H_{70}N_2O_{15}$: C, 66.52; H, 6.98; N 2.77. Found: C, 66.64; H, 6.83; N, 2.62.

(b) A mixture of thioglycoside **28** (1.25 g, 2.50 mmol), 5-methoxycarbonylpentyl 3-*O*-benzyl-4-(2,4-*O*-benzylidene-3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-α-D-mannopyranoside[15] (1.2 g, 2.10 mmol) and 4 Å molecular sieves (6 g) in dry CH_2Cl_2 (30 mL) was stirred for 15 min at room temperature. NIS (0.75 g, 3.33 mmol) was added, followed by solid AgOTf (0.23 g, 0.92 mmol). The mixture turned red after few minutes, the

stirring was continued for another 15 min, when the reaction was quenched by the addition of Et_3N (1 mL). The mixture was partitioned between aq 1:1 $Na_2S_2O_3$–$NaHCO_3$, the organic layer was dried with $MgSO_4$, and concentrated. Chromatography (5:1 → 2:1 toluene–acetone) gave material (1.8 g, 85%), which was identical (TLC, NMR) with compound **7** described above.

3.6. 5-Methoxycarbonylpentyl 4-(3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-2-*O*-methyl-α-D-mannopyranosyl-(1 → 2)-4-(3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-α-D-mannopyranoside (11)

A mixture of **7** (1.0 g, 0.99 mmol) and 5% palladium-on-carbon catalyst (600 mg) in methanol (50 mL) was stirred under hydrogen overnight, when TLC (4:1 CH_2Cl_2–MeOH) showed that the reaction was complete. Conventional processing and elution from a small column of silica gel (6:1 to 3:1 CH_2Cl_2–MeOH) gave amorphous, deprotected methyl ester **11** (592 mg, 92%); $[\alpha]_D$ −15.1° (*c* 0.5, CH_3OH). Definite signals in the ^1H NMR (D_2O) spectrum were at: δ 5.17 (d, 1 H, $J_{1,2}$ 1.6 Hz, H-1^{II}), 4.92 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1^{I}), 4.30, 4.29 (2 dd, partially overlapped, 2 H, H-$2'^{I,II}$), 4.10 (dd, partially overlapped, $J_{2,3}$ 3.3, $J_{3,4}$ 10.3 Hz, H-3^{II}), 4.06 (dd, partially overlapped, $J_{2,3}$ 3.3, $J_{3,4}$ 10.3 Hz, H-3^{I}), 3.96 (broad t, partially overlapped, H-2^{I}), 3.95–3.67 (m, 13 H, H-$4^{I,II}$,$5^{I,II}$,$4'^{I,II}$,1″a, incl dd, at 3.77, partially overlapped, for H-2^{II}, and s, at 3.69 for $COOCH_3$), 3.58–3.50 (m, 1 H, H-1″b), 3.49 (s, 3 H, OCH_3-2), 2.41 (t, 2 H, *J* 7.5 Hz, H-5″a,b), 2.10–1.80 (2 m, 4 H, H-$3'^{I,II}$a,b), 1.69–1.58 (m, 4 H, H-2″a,b,4″a,b), 1.44–1.35 (H-3″a,b), 1.20, 1.17 (2 d, partially overlapped, 6 H, H-$6^{I,II}$); ^{13}C NMR (D_2O): δ 99.22 (C-1^{II}), 98.47 (C-1^{I}), 79.06 (C-2^{II}), 78.36 (C-2^{I}), 69.09 (2 C, C-$2'^{I,II}$), 68.30 (2 C, C-5,1″), 67.71, 67.68, 67.58 (3 C, C-$5,3^{I,II}$), 58.86 (OCH_3-2), 57.94 (2 C, C-$4'^{I,II}$), 53.29, 53.18 (C-$4^{I,II}$), 52.22 ($COOCH_3$), 36.09, 36.06 (C-$3'^{I,II}$), 33.70 (C-5″), 28.19 (C-2″), 24.98 (C-3″), 24.10 (C-4″), 16.97. 16.92 (C-$6^{I,II}$); FABMS: *m*/*z* 655.4 ($[M+1]^+$), 677.4 ($[M+Na]^+$); HRMS: *m*/*z* 661.3367. $C_{28}H_{50}N_2O_{15}Li$ requires 661.3371.

3.7. (2-Aminoethylamido)carbonylpentyl 4-(3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-2-*O*-methyl-α-D-mannopyranosyl-(1 → 2)-4-(3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-α-D-mannopyranoside (15)

Following the general procedure described above, conversion of **11** (270 mg) gave the amine **15** (229 mg, 85%); ^1H NMR (CD_3OD): δ 5.09 (d, 1 H, $J_{1,2}$ 1.6 Hz, H-1^{II}), 4.82 (d, 1 H, $J_{1,2}$ 1.6 Hz, H-1^{I}), 4.22–4.15 (m, 2 H, H-$2'^{I,II}$), 3.98 (dd, partially overlapped, $J_{2,3}$ 3.1, $J_{3,4}$ 10.2 Hz, H-3^{I}), 3.98–3.65 (m, 12 H, H-3^{II},$4^{I,II}$,$5^{I,II}$, 4′a,b^{I,II},1″a, incl. broad, ~3.82, H-2^{I}, broad, ~3.68, H-2^{II}), 3.48 (s, 3 H, OCH_3), 3.45–3.38 (m, 1 H, H-1″b), 3.29 (t, 2 H, *J* 6.3 Hz, H-6″a,b), 2.78 (t, 2 H, *J* 7.4 Hz, H-7″a,b), 2.23 (t, *J* 7.8 Hz, H-5″a,b), 2.08–1.77 (2 m, 4 H, H-$3'^{I,II}$a,b), 1.70–1.57 (m, 4 H, H-4″a,b,2″a,b), 1.46–1.34 (m, 2 H, H-3″a,b), 1.17, 1.14 (2 d, $J_{5,6}$ 6.2 Hz, H-$6^{I,II}$). The ^1H NMR spectrum taken in pyridine-d_5 showed a 3-proton multiplet (δ 8.1–7.90) for the three NH groups; ^{13}C NMR (CD_3OD): δ 100.79 (C-1^{II}), 100.25 (C-1^{I}), 80.69 (C-2^{II}), 79.68 (C-2^{I}), 70.64 (2 C, C-$2'^{I,II}$), 69.64, 69.57 (C-$3^{I,II}$), 69.31, 68.73 (C-$5^{I,II}$), 68.46 (C-1″), 59.40, 59.38 (C-$4'^{I,II}$), 59.19 (OCH_3), 54.72, 54.48 (C-$4^{I,II}$), 42.07 (C-6″), 41.78 (C-7″), 38.25 (2 C, C-$3'^{I,II}$), 36.94 (C-5″), 30.15 (C-2″), 26.87 (C-3″), 26.16 (C-4″), 18.36, 18.27 (C-$6^{I,II}$); FABMS: *m*/*z* 683.38 ($[M+1]^+$), 705.38 ($[M+Na]^+$); HRMS: *m*/*z* 689.3768. $C_{29}H_{54}N_4O_{14}Li$ requires 689.3797.

3.8. 1-{(2-Aminoethylamido)carbonylpentyl 4-(3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-2-*O*-methyl-α-D-mannopyranosyl-(1 → 2)-4-(3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-α-D-mannopyranoside}-2-ethoxycyclobutene-3,4-dione (19)

Following the general procedure described above, conversion of **15** (50 mg) gave the ethyl ester **19** (57 mg, 96%). Definite signals in the ^1H NMR spectrum (CD_3OD) were at δ 5.09 (d, 1 H, $J_{1,2}$ 1.6 Hz, H-1^{II}), 4.83 (d, 1 H, $J_{1,2}$ 1.6 Hz, H-1^{I}), 4.78–4.68 (m, 2 H, OCH_2CH_3), 4.21–4.16 (m, 2 H, H-$2'^{I,II}$), ~3.82 (broad, partially overlapped, H-2^{I}), ~3.74 (m, partially overlapped, $4'^{I,II}$a,b), 3.68 (broad, partially overlapped, H-2^{II}), 3.48 (s, 3 H, OCH_3), 2.23–2.15 (2 t, partially overlapped, 2 H, H-5″a,b), 2.08–1.77 (m, 4 H, H-$3'^{I,II}$a,b), 1.66–1.54 (m, 4 H, H-4″a,b,2″a,b), 1.49–1.34 (m, 5 H, CH_3CH_2, H-3″a,b), 1.17, 1.14 (2 d, $J_{5,6}$ 6.2 Hz, H-$6^{I,II}$); ^{13}C NMR (CD_3OD): δ 100.80 (C-1^{II}), 100.24 (C-1^{I}), 80.68 (C-2^{II}), 79.64 (C-2^{I}), 70.70 (d, CH_2CH_3), 70.69 (2 C, C-$2'^{I,II}$), 69.66, 69.61 (C-$3^{I,II}$), 69.33, 68.73 (C-$5^{I,II}$), 68.44 (C-1″), 59.44, 59.41 (C-$4'^{I,II}$), 59.21 (OCH_3), 54.73, 54.50 (C-$4^{I,II}$), 45.20 (d, C-6″), 40.75 (d, C-7″), 38.24, 38.21 (C-$3'^{I,II}$), 36.92 (C-5″), 30.18 (C-2″), 26.82 (d, C-3″), 26.55 (d, C-4″), 18.34, 18.26 (C-$6^{I,II}$), 16.17 (d, CH_3CH_2); FABMS: *m*/*z* 807 ($[M+1]^+$).

3.9. 5-Methoxycarbonylpentyl 3-*O*-benzyl-4-(2,4-*O*-benzylidene-3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-2-*O*-methyl-α-D-mannopyranosyl-(1 → 2)-3-*O*-benzyl-4-(2,4-*O*-benzylidene-3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-α-D-mannopyranosyl-(1 → 2)-3-*O*-benzyl-4-(2,4-*O*-benzylidene-3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-α-D-mannopyranoside (8)

(a) The trisaccharide alcohol **4** (320 mg) was treated for 3 days with MeI (7 mL) and Ag_2O (5 g), as described for the preparation of **7** (a). Chromatography (5:1 to 2:1 toluene–acetone) gave the desired, fully protected methyl ether **8** (290 mg, 90%), $[\alpha]_D$ −23.3° (*c* 0.45);

^{1}H NMR ($CDCl_3$): δ 6.31, 6.25 (d, 2 H, d, 1 H, $J_{4,NH}$ 9.3 Hz, 3 NH), 5.56, 5.53, 5.52 (3 s, 1 H each, 3 C*H*Ph), 5.00 (d, 1 H, $J_{1,2}$ 1.7 Hz, H-1^{III}), 4.95 (d, 1 H, $J_{1,2}$ 1.8 Hz, H-1^{II}), 4.70–4.27 (m, 13 H, 3 CH_2Ph, 2′$^{I\text{-}III}$,4′a$^{I\text{-}III}$, incl. d, overlapped, H-1^I), 4.19 (broad t, 1 H, H-2^{II}), 4.50–3.92 (m, 6 H, H-4^{I-III},4′$^{I-III}$), 3.87 (broad t, 1 H, H-2^I), 3.78–3.67 (m, 7 H, H-2^{II},3^{I-III},5^{I-III}), 3.60–3.53 (m, 4 H, H-1″a, incl s at 3.59, $COOCH_3$), 3.33–3.25 (m, 4 H, H-1″b, incl s at 3.59, OCH_3-2), 2.27 (t, 2 H, *J* 7.5 Hz, H-5″a,b), 2.10–1.79 (m, 6 H, H-3^{I-III}a,b), 1.65–1.48 (m, 4 H, H-2″a,b,4″a,b), 1.35–1.24 (m, 2 H, H-3″a,b), 1.18, 1.16, 1.07 (3 d, partially overlapped, $J_{5,6}$ 6.2 Hz, H-6^{I-III}); ^{13}C NMR ($CDCl_3$): δ 101.36, 101.19 (C, 2 C, 3 *C*HPh), 101.11 (C-1^{II}), 99.14 (C-1^{III}), 98.86 (C-1^I), 76.57, 76.50 (2 C, C, C-2′$^{I-III}$), 75.72 (2 C, C-2^{III},3), 75.35, 75.28 (2 C-3), 74.33 (C-2^I), 72.71 (C-2^{II}), 71.98, 71.43, 70.98 (3 *C*H$_2$Ph), 68.45, 68.42, 67.48 (C-5^{I-III}), 67.31, 67.27, 67.22 (C, C, 2 C, C-1″,4′$^{I-III}$), 59.03 (OCH_3-2), 52.25, 51.94, 51.83 (C-4^{I-III}), 51.38 ($COO*C*H_3$), 33.81 (C-5″), 28.78 (C-2″), 28.52 (3 C, C-3′$^{I-III}$), 25.49 (C-3″), 24.48 (C-4″), 18.02, 17.91 (C, 2 C, C-6^{I-III}); FABMS: *m*/*z* 1436.74 ([M+1]$^+$), 1458.72 ([M+Na]$^+$); HRMS: *m*/*z* 1568.5715. $C_{80}H_{97}N_3O_{21}Cs$ requires 1568.5669.

A small amount of by product, analogous to compound **29**, which was formed during methylation of **3**, was also obtained.

(b) A mixture of the glycosyl donor **28** (90 mg, 0.18 mmol) and glycosyl acceptor **3**[15] (120 mg, 0.12 mmol) in dry CH_2Cl_2 (10 mL) was treated with NIS and AgOTf (21 mg, 0.084 mmol), as described for the preparation of **7** (b). Chromatography (5:1 to 2:1 toluene–acetone) gave **8** (133 mg, 77%), which was identical (TLC, NMR) with the compound described above.

3.10. 5-Methoxycarbonylpentyl 4-(3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-2-*O*-methyl-α-D-mannopyranosyl-(1→2)-4-(3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-α-D-mannopyranosyl-(1→2)-4-(3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-α-D-mannopyranoside (12)

Treatment of **8** (900 mg) with hydrogen, as described for the preparation of **11**, gave **12** (540 mg, ~100%). Definite signals in the ^{1}H NMR spectrum (CD_3OD) were at δ 5.13–5.11 (m, 2 H, H-1^{II,III}), 4.82 (d, 1 H, $J_{1,2}$ 1.6 Hz, H-1^I), 4.22–4.16 (m, 3 H, H-2′$^{I-III}$), 4.12 (broad doublet, 1 H, H-2^{II}), 4.04 (dd, 1 H, $J_{2,3}$ 3.2, $J_{3,4}$ 10.2 Hz, H-3^{II}), 3.68–3.65 (m, 5 H, partially overlapped, H-2^{III},1″a, incl s at 3.67 for OCH_3-2), 3.48 (s, 3 H, $COOCH_3$), 3.45–3.38 (m, 1 H, H-1″b), 2.35 (t, 2 H, *J* 7.3 Hz, H-5″a,b), 2.08–1.77 (2 m, 6 H, H-3′a,b), 1.69–1.57 (m, 4 H, H-2″a,b,4″a,b), 1.46–1.38 (m, 2 H, H-3″a,b), 1.17, 1.16, 1.13 (3 d, 9 H, $J_{5,6}$ 6.1 Hz, H-6^{I-III}); ^{13}C NMR (CD_3OD): δ 102.68 (C-1^{II}), 100.43 (C-1^{III}), 100.15 (C-1^I), 80.65 (C-2^{III}), 79.86 (C-2^I), 78.90 (C-2^{II}), 70.66, 70.61 (C, 2 C, C-2′$^{I-III}$), 66.67 (C-3^{III}), 69.55 (C-3^I), 69.40 (C-5), 69.32 (2 C, C-3^{II},5), 68.72 (C-5), 68.37 (C-1″), 59.41, 59.37 (C, 2 C, C-4′$^{I-III}$), 59.13 (OCH_3-2), 54.69, 54.47 (C, 2 C, C-4′$^{I-III}$), 52.07 ($COO*C*H_3$), 38.21 (3 C, C-3′$^{I-III}$), 34.59 (C-5″), 30.00 (C-2″), 26.67 (C-3″), 25.56 (C-4″), 18.29, 18.14 (2 C, C, C-6^{I-III}). FABMS: *m*/*z* 1034.3 ([M+Cs]$^+$).

3.11. (2-Aminoethylamido)carbonylpentyl 4-(3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-2-*O*-methyl-α-D-mannopyranosyl-(1→2)-4-(3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-α-D-mannopyranosyl-(1→2)-4-(3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-α-D-mannopyranoside (16)

Following the general procedure described above, conversion of **12** (50 mg) gave the amine **16** (45 mg, 87%). Definite signals in the ^{1}H NMR spectrum (CD_3OD) were at δ 5.12 (bs, 2 H, H-1^{II-III}), 4.82 (bs, 1 H, H-1^I), 4.22–4.16 (m, 3 H, H-2′$^{I-III}$), 4.12 (broad t, 1 H, H-2^{II}), 4.04 (dd, 1 H, $J_{2,3}$ 3.1, $J_{3,4}$ 10.0 Hz, H-3^{II}), 3.67 (broad t, 1 H, H-2^{III}), 3.48 (s, partially overlapped, OCH_3-2), 3.28 (t, 1 H, *J* 6.5 Hz, H-6″a,b), 2.77 (t, 2 H, H-7″a,b), 2.23 (t, 2 H, *J* 7.2 Hz, H-5″), 2.08–1.77 (2 m, 6 H, H-3′$^{I-III}$), 1.70–1.57 (m, 4 H, H-4″a,b,2″a,b in that order), 1.46–1.36 (m, 2 H, H-3″a,b), 1.17, 1.16, 1.15 (3 d, 9 H, $J_{5,6}$ 5.8 Hz, H-6^{I-III}); ^{13}C NMR (CD_3OD): δ 102.68 (C-1^{II}), 100.44 (C-1^{III}), 100.17 (C-1^I), 80.68 (C-2^{III}), 79.87 (C-2^I), 78.91 (C-2^{II}), 70.63 (3 C, C-2′$^{I-III}$), 69.65 (C-3^{III}), 69.52 (C-3^I), 69.43 (C-5), 69.34 (C-3^{II},5), 68.74 (C-5), 68.45 (C-1″), 59.38 (3 C, C-4′$^{I-III}$), 59.17 (OCH_3), 54.76, 54.49 (C, 2 C, C-4′$^{I-III}$), 42.20 (C-6″), 41.80 (C-7″), 38.23 (3 C, C-3′$^{I-III}$), 36.94 (C-5″), 30.11 (C-2″), 26.84 (C-3″), 26.51 (C-4″), 18.34, 18.19 (2 C, C, C-6^{I-III}); FABMS: *m*/*z* 930.6 ([M+1]$^+$).

3.12. 1-[(2-Aminoethylamido)carbonylpentyl 4-(3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-2-*O*-methyl-α-D-mannopyranosyl-(1→2)-4-(3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-α-D-mannopyranosyl-(1→2)-4-(3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-α-D-mannopyranoside]-2-ethoxycyclobutene-3,4-dione (20)

Following the general procedure described above, conversion of **16** (50 mg) gave the ethyl ester **20** (36 mg, 65%). Definite signals in the ^{1}H NMR spectrum (CD_3OD) were at δ 5.12 (2 d, partially overlapped, 2 H, H-1^{II,III}), 4.82 (broad doublet, 1 H, H-1^I), 4.22–4.16 (m, 3 H, H-2′$^{I-III}$), 4.12 (dd, 1 H, $J_{1,2}$ 1.7, $J_{2,3}$ 3.0 Hz, H-2^{II}), 4.04 (dd, 1 H, $J_{3,4}$ 10.1 Hz, H-3^{II}), 3.67 (m, H-2^{III},6″a,b), 3.54–3.48 (m, 4 H, H-1″a, incl s, 3.48, OCH_3-2), 3.44–3.36 (m, 3 H, H-1″b,7″a,b), 2.22–2.14 (2 t, partially overlapped, 2 H, H-5″), 2.08–1.77 (2 m, 6 H, H-3′$^{I-III}$), 1.67–1.54 (m, 4 H, H-4″a,b,2″a,b in that order), 1.49–1.35 (m, 5 H, CH_3CH$_2$, H-3″a,b), 1.17, 1.15, 1.13 (3 d, 9 H, $J_{5,6}$ 6.0 Hz, H-6^{I-III}); ^{13}C NMR (CD_3OD): δ 102.69 (C-1^{II}), 100.49 (C-1^{III}), 100.19 (C-

1^{I}), 80.71 (C-2^{III}), 79.84 (C-2^{I}), 78.93 (C-2^{II}), 70.76 (d, CH_2CH_3), 70.72, 70.65 (2 C, C, C-$2'^{I-III}$), 69.71, 69.60, 69.45, 69.38, 68.75 (C, C, C, 2 C, C, C-3^{I-III},5^{I-III}), 68.38 (C-1″), 59.45, 59.42, 59.41 (C-$4'^{I-III}$), 59.16 (OCH_3-2), 54.79, 54.52 (C, 2 C, C-$4'^{I-III}$), 45.18 (d, C-6″), 40.75 (d, C-7″), 38.26, 38.22 (2 C, C, C-$3'^{I-III}$), 36.91 (C-5″), 30.17 (C-2″), 26.80 (d, C-3″), 26.47 (C-4″), 18.35, 18.19 (2 C, C, C-6^{I-III}), 16.15 (d, CH_3CH_2); FABMS: *m/z* 1054.4 ($[M+1]^+$).

3.13. 5-Methoxycarbonylpentyl 3-*O*-benzyl-4-(2,4-*O*-benzylidene-3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-2-*O*-methyl-α-D-mannopyranosyl-bis-[(1→2)-(3-*O*-benzyl-4-(2,4-*O*-benzylidene-3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-α-D-mannopyranosyl]-(1→2)-3-*O*-benzyl-4-(2,4-*O*-benzylidene-3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-α-D-mannopyranoside (9)

(a) The trisaccharide alcohol **4** (50 mg) was treated with MeI (2.5 mL) and Ag_2O (1.5 g), as described for the preparation of **7** (a). The reaction time had to be extended to 5 days in order for the starting alcohol to be almost completely consumed, as shown by TLC. Chromatography (5:1 to 2:1 toluene–acetone) gave the desired, fully protected methyl ether **9** (45 mg, 90%), $[\alpha]_D$ −29° (*c* 0.4). Definite signals in the ^{1}H NMR spectrum ($CDCl_3$) of **9** were at δ 6.34, 6.28, 6.27, 6.19 (4 d, partially overlapped, 4 H, $J_{4,NH}$ 9.4 Hz, 4 NH), 5.55, 5.54, 5.53, 5.52 (4 s, 4 C*H*Ph), 4.99 (d, 1 H, $J_{1,2}$ 1.8 Hz, H-1^{IV}), 4.97, 4.93 (2 d, 1 H each, $J_{1,2}$ 1.8 Hz, H-1^{II-III}), ~4.62 (part of a multiplet, H-1^{I}), 4.20, 4.10 (2 broad t, partially overlapped, H-$2^{II,III}$), 3.84 (broad t, partially overlapped, H-2^{I}), 3.78 (broad t, partially overlapped, H-2^{IV}), 3.59 (s, partially overlapped, $COOCH_3$), 3.26 (s, partially overlapped, OCH_3-2), 2.26 (t, 2 H, *J* 7.5 Hz, H-5″a,b), 2.06–1.84 (m, 8 H, H-$3'^{I-IV}$a,b), 1.64–1.47 (m, 4 H, H-2″,4″a,b), 1.34-1.25 (m, 2 H, H-3″a,b), 1.15, 1.13. 1.08. 1.05 (4 d, 12 C, H-6^{I-IV}); ^{13}C NMR ($CDCl_3$): δ 101.35, 101.24, 101.13 (C, C, 2 C, 4 *C*HPh), 100.90, 100.50 (C-$1^{II,III}$), 99.12 (C-1^{IV}), 98.78 (C-1^{I}), 76.64, 76.58, 76.50 (C, 2 C, C, C-$2'^{I-IV}$), 75.79 (C-2^{IV}), 75.68, 75.44, 75.24, 74.60 (C-3^{I-IV}), 74.16 (C-2^{I}), 72.92, 72.86 (C-$2^{II,III}$), 71.88, 71.34, 71.24, 71.00 (4 CH_2Ph), 68.59, 68.51, 68.42, 67.52 (C-5^{I-IV}), 67.27 (5 C, C-1″,$4'^{I-IV}$), 59.01 (OCH_3-2), 52.16, 52.00, 51.90, 51.63 (C-4^{I-IV}), 51.41 ($COOCH_3$), 33.82 (C-5″), 28.79 (C-2″), 28.54 (4 C, C-$3'^{I-IV}$), 25.49 (C-3″), 24.49 (C-4″), 18.03, 17.93 (C, 3 C, C-6^{I-IV}). FABMS: *m/z* 1861.93 ($[M+1]^+$), 1883.94 ($[M+Na]^+$); HRMS: *m/z* 1993.7477. $C_{104}H_{124}CsN_4O_{27}$ requires 1993.7507.

A small amount of by product, analogous to compound **29**, which was formed during methylation of **3**, was also obtained.

(b) Reaction of the glycosyl donor **28** (240 mg, 0.48 mmol), and alcohol **4**[15] (440 mg, 0.31 mmol), as described for the preparation of **7** (b), followed by chromatography (5:1→2:1 toluene–acetone) gave material (455 mg, 79%), which was identical (TLC, NMR) with the fully protected tetrasaccharide **9** described above.

3.14. 5-Methoxycarbonylpentyl 4-(3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-2-*O*-methyl-α-D-mannopyranosyl-bis-[(1→2)-4-(3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-α-D-mannopyranosyl]-(1→2)-4-(3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-α-D-mannopyranoside (13)

Hydrogenolysis of the fully protected tetrasaccharide **9** (700 mg), as described for the preparation of **8**, gave **13** (420 mg, ~100%). Definite signals in the ^{1}H NMR spectrum (CD_3OD) were at δ 5.14–5.11 (m, 3 H, (H-1^{II-IV}), 4.82 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1^{I}), 4.22–4.16 (m, 4 H, H-$2'^{I-IV}$), 4.12–4.08 (m, 2 H, H-$2^{II,III}$), 4.06–4.01 (m, 2 H, H-$3^{II,III}$), 3.68–3.65 (m, partially overlapped, H-2^{IV},1″a, incl. s at 3.66 for $COOCH_3$), 3.48 (s, 3 H, OCH_3-2), 3.45–3.38 (m, 1 H, H-1″b), 2.34 (t, 2 H, *J* 7.2 Hz, H-5″a,b), 2.08–1.76 (2 m, 6 H, H-3′a,b), 1.69–1.57 (m, 4 H, H-2″a,b,4″a,b), 1.46–1.38 (m, 2 H, H-3″a,b), 1.18–1.12 (m, 12 H, H-6^{I-IV}); ^{13}C NMR (CD_3OD): δ 102.69, 102.38 (C-$1^{II,III}$), 100.44 (C-1^{IV}), 100.16 (C-1^{I}), 80.64 (C-2^{IV}), 79.84 (C-2^{I}), 78.99 (2 C, C-$2^{II,III}$), 70.61 (4 C, C-$2'^{I-IV}$), 69.67 (C-3^{IV}), 69.57 (C-3^{I}), 69.42, 69.34 (2 C, 3 C, C-$3^{II,III}$,5,5,5), 68.71 (C-5), 68.38 (C-1″), 59.38 (4 C, C-$4'^{I-IV}$), 59.12 (OCH_3-2), 54.70, 54.48 (C, 3 C, C-$4'^{I-IV}$), 52.09 ($COOCH_3$), 38.21 (4 C, C-$3'^{I-IV}$), 34.60 (C-5″), 30.00 (C-2″), 26.68 (C-3″), 25.57 (C-4″), 18.31, 18.24, 18.18 (2 C, C, C, C-6^{I-IV}); FABMS: *m/z* 1281.4 ($[M+Cs]^+$).

3.15. (2-Aminoethylamido)carbonylpentyl 4-(3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-2-*O*-methyl-α-D-mannopyranosyl-bis-[(1→2)-4-(3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-α-D-mannopyranosyl]-(1→2)-(4-(3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-α-D-mannopyranoside (17)

Treatment of the methyl ester **13** (80 mg) as described for preparation of amine **16,** gave the title compound **17** (73 mg, 90%). Definite signals in the ^{1}H NMR spectrum (CD_3OD) were at δ 5.15 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1), 5.12 (m, 2 H, H-1), 4.81 (d, 1 H, $J_{1,2}$ 1.4 Hz, H-1^{I}), 4.22–4.15 (m, 4 H, H-$2'^{I-IV}$), 4.12–4.08 (m, 2 H, H-$2^{II,III}$), 4.06–4.01 (m, partially overlapped, 2 H, H-$3^{II,III}$), ~3.67 (m, partially overlapped, H-2^{IV}), 3.48–3.38 (m, 4 H, H-1″b, incl s, 3.48, OCH_3-2), 3.26 (t, 2 H, *J* 6.7 Hz, H-6″a,b), 2.73 (t, 2 H, H-7″a,b), 2.22 (t, 2 H, *J* 7.3 Hz, H-5″a,b), 2.08–1.76 (2 m, 8 H, H-$3'^{I-IV}$), 1.69–1.57 (m, 4 H, H-4″a,b,2″a,b in that order), 1.46–1.36 (m, 2 H, H-3″a,b), 1.18–1.13 (4 d, partially overlapped, 12 H, H-6^{I-IV}); ^{13}C NMR (CD_3OD): δ 102.71, 102.41 (C-$1^{II,III}$), 100.49 (C-1^{IV}), 100.19 (C-1^{I}), 80.68 (C-2^{IV}), 79.87 (C-2^{I}), 79.02 (2

C, C-$2^{II,III}$), 70.63 (4 C, C-$2'^{I-IV}$), 69.69 (C-3^{IV}), 69.57 (C-3^{I}), 69.46, 69.38, 68.74 (2 C, 3 C, C, C-$3^{II,III}$,5^{I-IV}), 68.47 (C-1″), 59.40 (4 C, C-$4'^{I-IV}$), 59.15 (OCH_3-2), 54.78, 54.51 (C, 3 C, C-$4'^{I-IV}$), 42.74 (C-6″), 41.95 (C-7″), 38.24 (C-$3'^{I-IV}$), 36.96 (C-5″), 30.12 (C-2″), 26.84 (C-3″), 26.54 (C-4″), 18.34, 18.26, 18.19 (2 C, C, C, C-6^{I-IV}); FABMS: *m/z* 1177 ($[M+1]^+$).

3.16. 1-{(2-Aminoethylamido)carbonylpentyl 4-(3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-2-*O*-methyl-α-D-mannopyranosyl-bis-[(1→2)-4-(3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-α-D-mannopyranosyl]-(1→2)-4-(3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-α-D-mannopyranoside}-2-ethoxycyclobutene-3,4-dione (21)

Treatment of amine **17** (70 mg) with squaric acid diethyl ester, as described above for similar conversions, gave the monoester **21** (76 mg, 96%). Definite signals in the ^{1}H NMR spectrum (CD_3OD) were at δ 5.15 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1^{II}), 5.12 (m, 2 H, 2 H-$1^{III,IV}$), 4.81 (d, 1 H, $J_{1,2}$ 1.4 Hz, H-1^{I}), 4.78–4.68 (m, 2 H, CH_2CH_3), 4.22–4.16 (m, 4 H, H-$2'^{I-IV}$), 4.12–4.08 (m, H-$2^{II,III}$), 4.06–4.01 (m, 2 H, H-$3^{II,III}$), ~3.67 (broad doublet of doublets, partially overlapped, H-2^{IV}), 3.54–3.47 (m, 4 H, H-1″a, incl s, 3.47, OCH_3-2), 3.43–3.37 (m, 3 H, H-1″b,7″a,b), 2.20, 2.17 (2 t, partially overlapped, 2 H, *J* 7.5 Hz, H-5″a,b), 2.08–1.76 (2 m, 8 H, H-$3'^{I-IV}$), 1.66–1.54 (m, 4 H, H-4″a,b,2″a,b in that order), 1.49–1.35 (m, 5 H, H-3″a,b, incl q at 1.46, CH_2CH_3), 1.18–1.13 (4 d, partially overlapped, 12 H, H-6^{I-IV}); ^{13}C NMR (CD_3OD): δ 102.63, 102.36 (C-$1^{II,III}$), 100.43 (C-1^{IV}), 100.15 (C-1^{I}), 80.65 (C-2^{IV}), 79.71 (C-2^{I}), 78.94 (2 C, C-$2^{II,III}$), 70.77 (d, CH_2CH_3), 70.63 (4 C, C-$2'^{I-IV}$), 69.66 (C-3^{IV}), 69.56 (C-3^{I}), 69.45, 69.34, 68.67 (2 C, 3 C, C, C-$3^{II,III}$,5^{I-IV}), 68.37 (C-1″), 59.41, 59.39 (2 C each, C-$4'^{I-IV}$), 59.13 (OCH_3-2), 54.75, 54.48 (C, 3 C, C-$4'^{I-IV}$), 45.16 (d, C-6″), 40.72 (d, C-7″), 38.20 (4 C, C-$3'^{I-IV}$), 36.88 (C-5″), 30.13 (C-2″), 26.79 (d, C-3″), 26.45 (d, C-4″), 18.34, 18.26, 18.19 (2 C, C, C, C-6^{I-IV}), 16.17 (d, CH_3CH_2); FABMS: *m/z* 1301.6 ($[M+1]^+$).

3.17. 5-Methoxycarbonylpentyl 3-*O*-benzyl-4-(2,4-*O*-benzylidene-3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-2-*O*-methyl-α-D-mannopyranosyl-tris-[(1→2)-3-*O*-benzyl-4-(2,4-*O*-benzylidene-3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-α-D-mannopyranosyl]-(1→2)-3-*O*-benzyl-4-(2,4-*O*-benzylidene-3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-α-D-mannopyranoside (10)

Reaction of the glycosyl donor **28** (252 mg, 0.50 mmol) with the tetrasaccharide glycosyl acceptor **6**,[15] as described for the preparation of **7** (b) gave the fully protected pentasaccharide **10** (575 mg, 75%), $[\alpha]_D$ −29° (*c* 0.5). Definite signals in the ^{1}H NMR spectrum ($CDCl_3$) of **10** were at δ 6.44–6.18 (5 d, partially overlapped, 5 H, 5 NH), 5.55, 5.54, 5.51 (3 s, 5 H, 5 C*H*Ph), 4.98 (d, 1 H, $J_{1,2}$ 1.8 Hz, H-1^{V}), 4.96, 4.89 (2 d, 3 H, $J_{1,2}$ 1.8 Hz, H-1^{II-IV}), ~4.61 (m, partially overlapped, H-1^{I}), 4.17–4.05 (m, partially overlapped, H-2^{II-IV}), 3.80 (broad doublet, partially overlapped, H-2^{I}), 3.77 (m, partially overlapped, H-2^{V}), 3.58 (s, partially overlapped, $COOCH_3$), 3.25 (s, partially overlapped, OCH_3-2), 2.26 (t, 2 H, *J* 7.5 Hz, H-5″a,b), 2.07–1.84 (m, 10 H, H-$3'^{I-V}$a,b), 1.64–1.47 (m, 4 H, H-2″,4″a,b), 1.34–1.25 (m, 2 H, H-3″a,b), 1.12, 1.07, 1.06, 1.03 (4 d, partially overlapped, 15 H, H-6^{I-IV}); ^{13}C NMR ($CDCl_3$): δ 101.32, 101.23 (3 C, 2 C, 5 *C*HPh), 100.99, 100.45 (C, 2 C, C-1^{II-IV}), 99.18 (C-1^{V}), 98.82 (C-1^{I}), 76.65, 76.60 (3 C, 2 C C-$2'^{I-V}$), 75.84 (2 C, C-2^{IV},3), 75.57, 75.21, 74.70, 74.50 (4 × C-3), 74.26 (C-2^{I}), 72.90, 72.78 (2 C, C, C-2^{II-IV}), 72.00, 71.30, 71.23, 71.10, 71.06 (5 CH_2Ph), 68.68, 68.63, 68.58, 67.62 (C, C, 2 C, C, C-5^{I-V}), 67.34 (6 C, C-1″,$4'^{I-V}$), 59.01 (OCH_3-2), 52.04, 51.98, 51.51, 51.40 (C, C, 2 C, C, C-4^{I-V}), 51.40 ($COOCH_3$), 33.88 (C-5″), 28.85 (C-2″), 28.61 (5 C, C-$3'^{I-V}$), 25.56 (C-3″), 24.55 (C-4″), 18.09, 18.01 (C, 4 C, C-6^{I-IV}); FABMS: *m/z* 2288.59 ($[M+1]^+$), 2310.81 ($[M+Na]^+$); HRMS: *m/z* 2418.9312. $C_{128}H_{151}CsN_5O_{33}$ requires 2418.9346.

3.18. 5-Methoxycarbonylpentyl 4-(3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-2-*O*-methyl-α-D-mannopyranosyl-tris-[(1→2)-(4-(3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-α-D-mannopyranosyl]-(1→2)-4-(3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-α-D-mannopyranoside (14)

Hydrogenolysis of the foregoing, fully protected pentasaccharide **10** (1 g), as described for the preparation of **11**, gave the methyl ester **14** (0.6 g, ~100%). Definite signals in the ^{1}H NMR spectrum (CD_3OD) were at δ 5.13, 5.11 (2 m, 4 H, (H-1^{II-V}), 4.81 (bs, 1 H, Hz, H-1^{I}), 3.67 (s, partially overlapped, $COOCH_3$), 3.47 (s, 3 H, OCH_3-2), 3.45–3.38 (m, 1 H, H-1″b), 2.35 (t, 2 H, *J* 7.2 Hz, H-5″a,b), 2.08–1.76 (2 m, 6 H, H-3′a,b), 1.69–1.57 (m, 4 H, H-2″a,b,4″a,b), 1.46–1.38 (m, 2 H, H-3″a,b), 1.18–1.13 (m, 15 H, H-6^{I-V}); ^{13}C NMR (CD_3OD): δ 102.64, 102.36 (C, 2 C, C-1^{II-IV}), 100.38 (C-1^{V}), 100.14 (C-1^{I}), 80.60 (C-2^{V}), 79.70 (C-2^{I}), 79.03, 78.90 (2 C, C-2^{II-IV}), 70.59 (5 C, C-$2'^{I-V}$), 69.29 (C-3^{V}), 69.55 (C-3^{I}), 69.38, 69.34 (2 C, 4 C, C-3^{II-IV},5,5,5,5), 68.69 (C-5), 68.37 (C-1″), 59.37 (5 C, C-$4'^{I-V}$), 59.12 (OCH_3-2), 54.65, 54.50, 54.44 (C, 2 C, 2 C, C-$4'^{I-V}$), 52.10 ($COOCH_3$), 38.16 (5 C, C-$3'^{I-V}$), 34.59 (C-5″), 29.97 (C-2″), 26.65 (C-3″), 25.55 (C-4″), 18.31, 18.27, 18.23, 18.19 (2 C, C, C, C, C-6^{I-V}); FABMS: *m/z* 1528.1 ($[M+Cs]^+$).

3.19. (2-Aminoethylamido)carbonylpentyl 4-(3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-2-*O*-methyl-α-D-mannopyranosyl-tris-[(1→2)-4-(3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-α-D-mannopyranosyl]-(1→2)-(4-(3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-α-D-mannopyranoside (18)

Treatment of the foregoing ester **14** (100 mg), as described for the preparation of **15**, gave amine **18** (85 mg, 83%). Definite signals in the ^{1}H NMR spectrum (CD_3OD) were at δ 5.14, 5.11 (2 m, 4 H, H-1^{II-V}), 4.81 (bs, 1 H, H-1^{I}), 4.22–4.16 (m, 5 H, H-2′$^{I-V}$), 3.67 (broad t, partially overlapped, H-2^{V}), 3.47–3.38 (m, 4 H, H-1″b, incl s, 3.47, OCH_3-2), 3.26 (t, 1 H, *J* 6.7 Hz, H-6″a,b), 2.73 (t, 2 H, *J* 6.3 Hz, H-7″a,b), 2.22 (t, 2 H, *J* 7.3 Hz, H-5″a,b), 2.08–1.76 (2 m, 10 H, H-3′$^{I-V}$), 1.70–1.57 (m, 4 H, H-4″a,b,2″a,b in that order), 1.46–1.36 (m, 2 H, H-3″a,b), 1.18–1.13 (5 d, partially overlapped, 15 H, H-6^{I-V}); ^{13}C NMR (CD_3OD): δ 102.70, 102.44 (C, 2 C, C-1^{II-IV}), 100.48 (C-1^{V}), 100.20 (C-1^{I}), 80.69 (C-2^{V}), 79.80 (C-2^{I}), 79.14, 79.01 (C, 2 C, C-2^{II-IV}), 70.64 (5 C, C-2′$^{I-V}$), 69.70 (C-3^{V}), 69.59 (C-3^{I}), 69.38, 68.74 (7 C, C, C-3^{II-IV},5^{I-V}), 68.47 (C-1″), 59.42 (5 C, C-4′$^{I-V}$), 59.15 (OCH_3), 54.76, 54.57, 54.51 (C, 2 C, 2 C, C-4^{I-V}), 42.71 (C-6″), 41.94 (C-7″), 38.25 (5 C, C-3′$^{I-V}$), 36.96 (C-5″), 30.12 (C-2″), 26.85 (C-3″), 26.54 (C-4″), 18.35, 18.29, 18.22 (2 C, 2 C, C, C-6^{I-V}); FABMS: *m*/*z* 1425 ([M+1]$^+$).

3.20. 1-{(2-Aminoethylamido)carbonylpentyl 4-(3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-2-*O*-methyl-α-D-mannopyranosyl-tris-[(1→2)-4-(3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-α-D-mannopyranosyl]-(1→2)-4-(3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-α-D-mannopyranoside}-2-ethoxycyclobutene-3,4-dione (22)

Treatment of amine **18** (85 mg) with diethyl squarate, as described for **15**, afforded the squaric acid ethyl ester **22** (83 mg, 89%). Definite signals in the ^{1}H NMR spectrum (CD_3OD) were at δ 5.14, 5.11 (2 m, 4 H, H-1^{II-V}), 4.80 (bs, 1 H, H-1^{I}), 4.73 (m, 2 H, CH_3CH_2), 4.22–4.16 (m, 5 H, H-2′$^{I-V}$), 3.67 (broad t, partially overlapped, 2^{IV}), 3.54–3.47 (m, 4 H, H-1″a, incl s, 3.47, OCH_3-2), 3.43–3.37 (m, 3 H, H-1″b,7″a,b), 2.20, 2.17 (2 t, partially overlapped, 2 H, J 7.3 Hz, H-5″a,b), 2.08–1.76 (2 m, 10 H, H-3′$^{I-V}$), 1.65–1.53 (m, 4 H, H-4″a,b,2″a,b in that order), 1.49–1.33 (m, 5 H, H-3″a,b, CH_3CH_2), 1.18–1.13 (5 d, partially overlapped, 15 H, H-6^{I-V}); ^{13}C NMR (CD_3OD): δ 102.62 (C-1^{III}), 102.39 (2 C, C-1^{II,IV}), 100.42 (C-1^{V}), 100.16 (C-1^{I}), 80.64 (C-2^{V}), 79.65 (C-2^{I}), 79.06, 78.92 (C, 2 C, C-2^{II-IV}), 70.79 (d, CH_3CH_2), 70.63 (5 C, C-2′$^{I-V}$), 69.66 (C-3^{V}), 69.57 (C-3^{I}), 69.38, 68.69 (7 C, C, C-3^{II-IV},5^{I-V}), 68.38 (C-1″), 59.42 (5 C, C-4′$^{I-V}$), 59.14 (OCH_3-2), 54.73, 54.55, 54.48 (C, 2 C, 2 C, C-4′$^{I-V}$), 45.17 (d, C-6″), 40.72 (d, C-7″), 38.20 (5 C, C-3′$^{I-V}$), 36.88 (C-5″), 30.13 (C-2″), 26.75 (d, C-3″), 26.45 (d, C-4″), 18.35, 18.28, 18.21 (3 C, C, C, C-6^{I-V}), 16.16 (d, CH_3CH_2); FABMS: *m*/*z* 1548.7 ([M+1]$^+$).

3.21. Ethyl 3-*O*-benzyl-4-(2,4-*O*-benzylidene-3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-2-*O*-methyl-1-thio-α-D-mannopyranoside (28)

Ethyl 2-*O*-acetyl-3-*O*-benzyl-4-(2,4-*O*-benzylidene-3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-1-thio-α-D-mannopyranoside[15] (2.5 g) was deacetylated (Zemplén). Conventional processing afforded pure (TLC) ethyl 3-*O*-benzyl-4-(2,4-*O*-benzylidene-3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-1-thio-α-D-mannopyranoside (**27**) (2.3 g, ~100%) as an oil; ^{1}H NMR ($CDCl_3$): δ 6.27 (broad doublet, 1 H, $J_{4,NH}$, NH), 5.56 (s, 1 H, C*H*Ph), 5.34 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1), 4.65, 4.50 (2 d, 2 H, ^2J 11.7 Hz, CH_2Ph), 4.40–4.32 (m, 2 H, H-2′,4′a), 4.13–3.27 (m, 4 H, H-2,4,5,4′b), 3.65 (dd, 1 H, $J_{2,3}$ 3.6, $J_{3,4}$ 10.2 Hz, H-3), 2.69–2.48 (m, 2 H, CH_2CH_3), 2.13–1.83 (2 m, 2 H, H-3′a,b), 1.27 (t, 1 H, *J* 7.5 Hz, CH_3CH_2), 1.23 (d, 3 H, $J_{5,6}$ 6.0 Hz, H-6); ^{13}C NMR ($CDCl_3$): δ 101.18 (*C*HPh), 83.01 (C-1), 76.44 (2 C, C-2′,3), 71.38 CH_2Ph), 68.53(C-5), 67.50 (C-2), 67.26 (C-4′), 51.90 (C-4), 28.56 (C-3′), 24.93 (CH_2CH_3), 17.76 (C-6), 14.79 (CH_2CH_3).

Silver oxide (15 g) was added to a soln of **27** (5.0 g, 10.3 mmol) in MeI (25 mL), and the mixture was stirred at r.t. with the exclusion of light. After 2 h, TLC (2:1 toluene–acetone) showed that no starting material was present. The reaction mixture was filtered, the filtrate was concentrated, and the residue was chromatographed (5:1→2:1 toluene–acetone), to give **28** (4.8 g, 93%), m.p. 97.5–98.0 °C (from isopropyl ether), $[\alpha]_D$ +32.6° (*c* 0.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 6.29 (d, 1 H, $J_{4,NH}$ 9.0 Hz, NH), 5.55 (s, 1 H, C*H*Ph), 5.36 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1), 4.65, 4.50 (2 d, 2 H, CH_2Ph), 4.36–4.30 (m, 2 H, H-2′,4′a), 4.19–3.95 (m, 3 H, H-4,5,4′b), 3.76 (dd, 1 H, $J_{2,3}$ 2.8, $J_{3,4}$ 10.2 Hz, H-3), 3.63 (dd, 1 H, H-2), 3.49 (s, 3 H, OCH_3), 2.70–2.51 (m, 2 H, CH_2CH_3), 2.09–1.81 (m, 2 H, H-3′a,b), 1.27 (t, 3 H, *J* 7.4 Hz, CH_2CH_3), 1.22 (d, 3 H, $J_{5,6}$ 5.9 Hz, H-6); ^{13}C NMR ($CDCl_3$): δ 101.02 (*C*HPh), 81.49 (C-1), 78.04 (C-2), 76.49 (C-2′), 76.27 (C-3), 71.04 (CH_2Ph), 67.84 (C-5), 67.25 (C-4′), 58.65 (OCH_3-2), 52.72 (C-4), 28.54 (C-3′), 25.36 (CH_2CH_3), 17.88 (C-6), 14.87 (CH_2CH_3); FABMS: *m*/*z* 502.31 ([M+1]$^+$), 524.31 ([M+Na]$^+$). Anal. Calcd for $C_{27}H_{35}NO_6S$: C, 64.65; H, 7.03; N, 2.79. Found: C, 64.55; H, 7.01; N, 2.86.

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