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Enhanced stereoselectivity of α-mannosylation under thermodynamic control using trichloroacetimidates

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ABSTRACT

O-Specific polysaccharides of *Vibrio cholerae* O1, serotypes Inaba and Ogawa, consist of α-(1→2)-linked *N*-(3-deoxy-L-*glycero*-tetronyl)perosamine (4-amino-4,6-dideoxy-D-mannose). The blockwise synthesis of larger fragments of such O-PSs involves oligosaccharide glycosyl donors that contain a nonparticipating 2-*O*-glycosyl group at the position vicinal to the anomeric center where the new glycosidic linkage is formed. Such glycosyl donors may bear at C-4 either a latent acylamino (e.g., azido) or the 3-deoxy-L-*glycero*-tetronamido group. While monosaccharide glycosyl donors, even those bearing a nonparticipating group at *O*-2 (e.g., methyl), *and* the 4-*N*-(3-deoxy-L-*glycero*-tetronyl) side chain form α-linked oligosaccharides with excellent stereoselectivity, α-mannosylation with analogous oligosaccharide donors in this series is adversely affected by the presence of the side chain. Consequently, the unwanted β-product is formed in a considerable amount. Conducting the reaction at elevated temperature under thermodynamic control substantially enhances formation of the α-linked oligosaccharide. This effect is much more pronounced when glycosyl trichloroacetimidates, rather than thioglycosides or glycosyl chlorides, are used as glycosyl donors.

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1. Introduction

Cholera in humans is caused by three strains of *Vibrio cholerae*—O1 Inaba, O1 Ogawa, and O139.[1] Progress in the development and practical relevance of a conjugate vaccine for cholera from a synthetic antigen depends, among other things, on the availability of oligosaccharides that mimic the O-specific polysaccharides (O-PSs) of the bacterial pathogens involved. The O-PSs of O1 Inaba and O1 Ogawa are very similar and consist of a (1→2)-α-linked perosamine (4-amino-4,6-dideoxy-D-mannose) whose amino group is acylated with 3-deoxy-L-*glycero*-tetronic acid. They differ[2,3] in that the Ogawa O-PS has a methyl group at O-2 of the upstream,[4] terminal perosamine residue (Fig. 1). We have been involved in the synthesis of oligosaccharides that mimic the O-PS of *V. cholerae* O1 and O139 for more than a decade.[5] The chemical syntheses are challenging and, despite several attempts to improve early approaches,[6–9] there is still a need to optimize the synthetic strategy. We have shown[10] that immunization of mice with a conjugate made from the synthetic hexasaccharide that mimics the upstream terminus of the Ogawa O-PS conferred protection. Therefore, we have recently focused our efforts on improving the synthesis of that segment in the *V. cholerae* O1 series.

Our recent blockwise synthesis[11] of the Ogawa hexasaccharide from disaccharide glycosyl donors bearing the 4-(3-deoxy-L-*glycero*-tetronamido) group in place (fully assembled glycosyl donors, as opposed to donors bearing 4-azido groups) has definite advantages over previous approaches, despite less than optimum stereoselectivity in the formation of the α-(1→2)-interglycosidic linkages. There,[11] we were able to improve the yield of the desired, α-linked product by reacting a disaccharide thioglycoside donor under thermodynamic control. Here we report on further improvement of the synthesis of the hexasaccharide sequence, which was accomplished by the use of relevant mono- and disaccharide

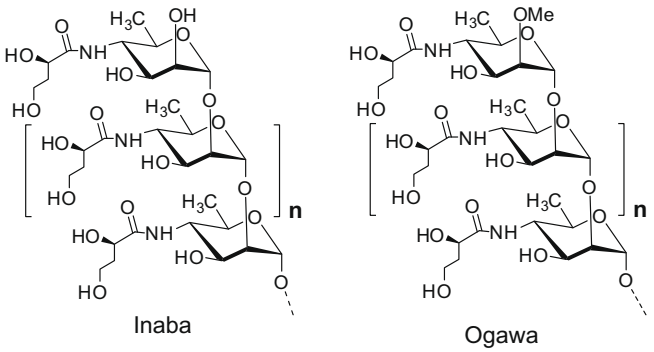

Figure 1. Structure of the O-PSs of the two strains of *Vibrio cholerae* O1.

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trichloroacetimidates as glycosyl donors. Under thermodynamic control, the stereoselectivity of formation of the α-mannosyl linkage markedly increased, compared to the use of thioglycosides as donors.[11]

2. Results and discussion

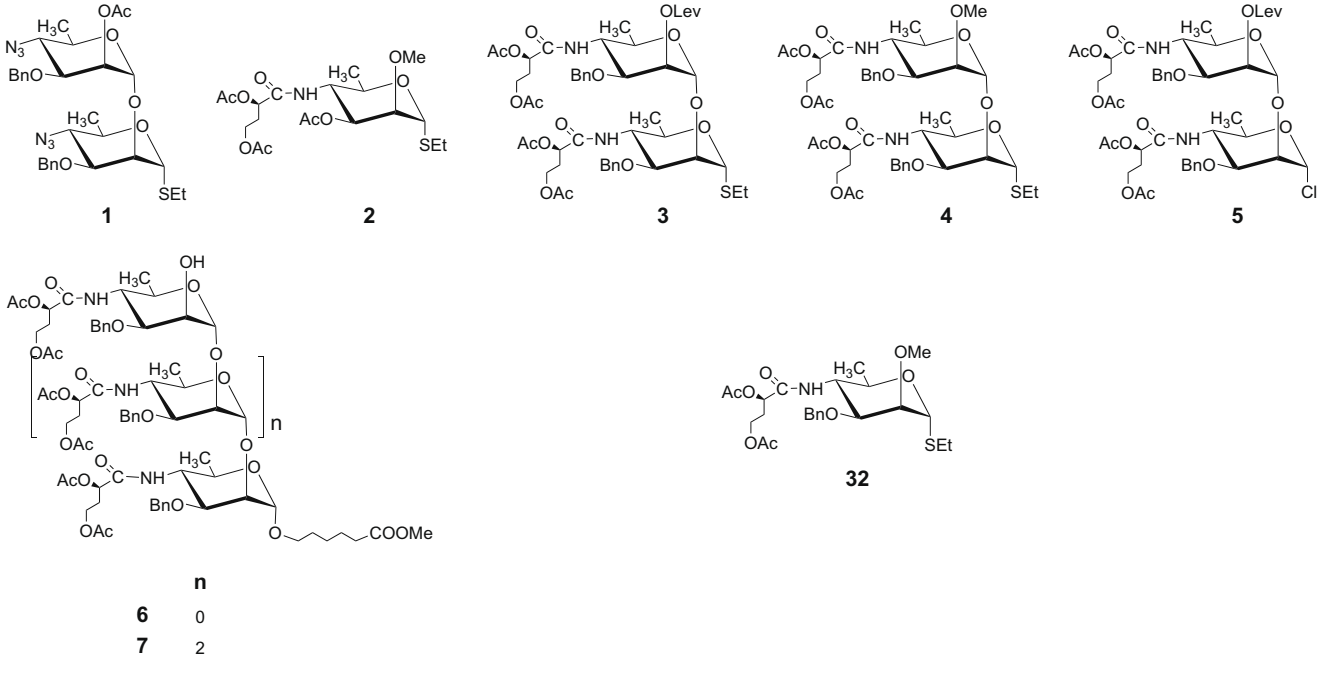
Generally, it is more efficient to synthesize higher oligosaccharides using a convergent (blockwise) strategy than by a linear (stepwise) approach. Also, in the assembly of large *N*-acyl-hexosamine-containing oligosaccharides, it is preferable to use intermediates where the *N*-acyl group is already installed (e.g., **3**–**5**) than those containing latent acylamino groups (e.g., **1**, where the azido group can be converted to an acylamino function at a later stage of the synthesis). Examples of such strategies can be found in the two different approaches to the tetrasaccharide side chain of the major glycoprotein of the *Bacillus anthracis* exosporium.[12,13] In our initial attempt to synthesize oligosaccharides in the *V. cholerae* O1 series,[14] we explored the feasibility of using fully assembled intermediates (cf., our synthesis of the Inaba disaccharide).[14] There, the formation of the α-mannopyranosyl linkage was highly stereoselective due to anchimeric assistance from a participating acyl group at *O*-2 in the monosaccharide glycosyl donor. This approach,[14] however, could not be extended to the synthesis of higher α-(1→2)-linked oligosaccharides because of the absence of a selectively removable protecting group in the product disaccharide. We have subsequently made a series of Inaba oligosaccharides by a stepwise approach from a fully assembled monosaccharide donor.[15] There, again, the stereoselectivity of glycosylation was not an issue because of the presence of the participating 2-*C*-acetyloxy group in the donor.

Seminal work by Peters and Bundle, within their synthetic work toward oligosaccharides that mimic the *Brucella* A polysaccharide, showed that large α-linked, perosamine-containing oligosaccharides can by synthesized in very good yields from donors that lack a participating moiety at C-2.[16,17] They used the C-4 azido group-containing (1→2)-linked disaccharide glycosyl donor **1** to synthesize α-mannopyranosyl linkages with high stereoselectivity (the pure α-products were obtained in >80% yields). Following their strategy, we have been able to prepare various oligosaccharides in the *V. cholerae* O1 series, including a dodecasaccharide.[7,18–20]

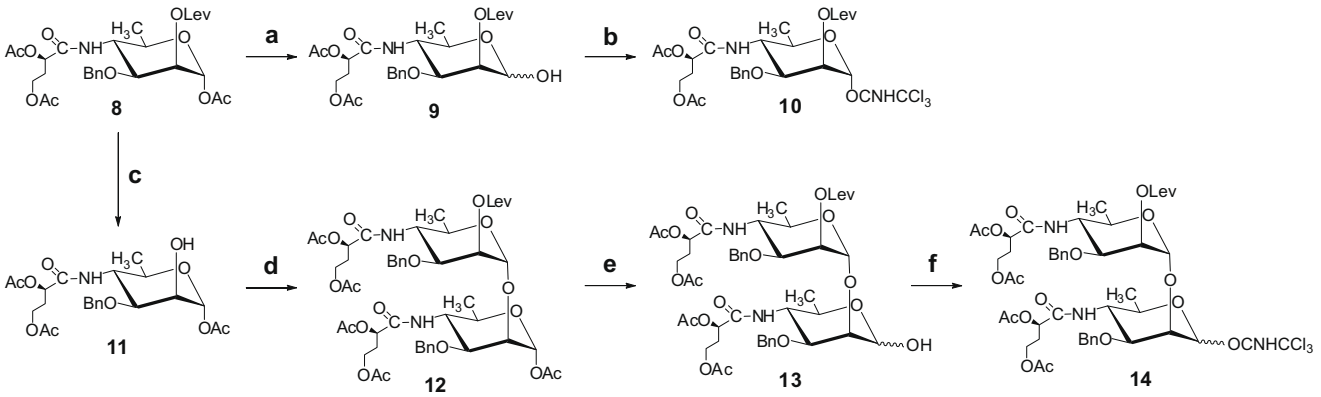
Encouraged by the high stereoselectivity of formation of the α-mannopyranosyl linkage in the absence of anchimeric assistance,[7,16,17] and by the precedence[21] for highly stereoselective formation of α-mannosyl linkage from the fully assembled donor **2**, we used[11] donors **3**–**5** for glycosylation in the assembly of the Ogawa hexasaccharide **27**. It turned out[11] that, unlike with glycosyl donors **1** and **2**, the presence of the side chain in the *oligosaccharide* donors **3**–**5** resulted in loss of the ability of these donors to form α-mannosyl linkage with high stereoselectivity. For example,[11] the reaction of thioglycoside **4** with methyl 6-hydroxyhexanoate in DCM at −20 °C gave mainly the unwanted β glycoside. Thus, paradoxically, although the synthesis of the β-mannosyl linkage is one of the most difficult glycosidic linkages to synthesize, we faced the uncommon task to minimize formation of that linkage. Conducting the glycosidation of methyl 6-hydroxyhexanoate at higher temperatures changed the situation considerably, as it resulted in increased relative amount of the desired α product formed, with the latter slightly predominating when the reaction was conducted at the temperature of refluxing toluene.[11] With oligosaccharide glycosyl acceptors **6** and **7** and donor **4**, the α:β ratio of products could be increased from 2:1 (DCM as solvent, room temperature) to 5:1 (refluxing toluene).[11]

A high yield of the α product is the prerequisite for efficient syntheses of *V. cholerae* O1 antigens. Guided by the increase of α-stereoselectivity of glycosylation under thermodynamic control using thioglycosides as glycosyl donors, we deemed it important to examine how trichloroacetimidate **14**, which is analogous to thioglycoside **3**,[11] would perform under similar conditions. To our knowledge, glycosylation under thermodynamic control with trichloroacetimidates has not been attempted, lest decomposition of the highly reactive donor might preclude glycosylation.

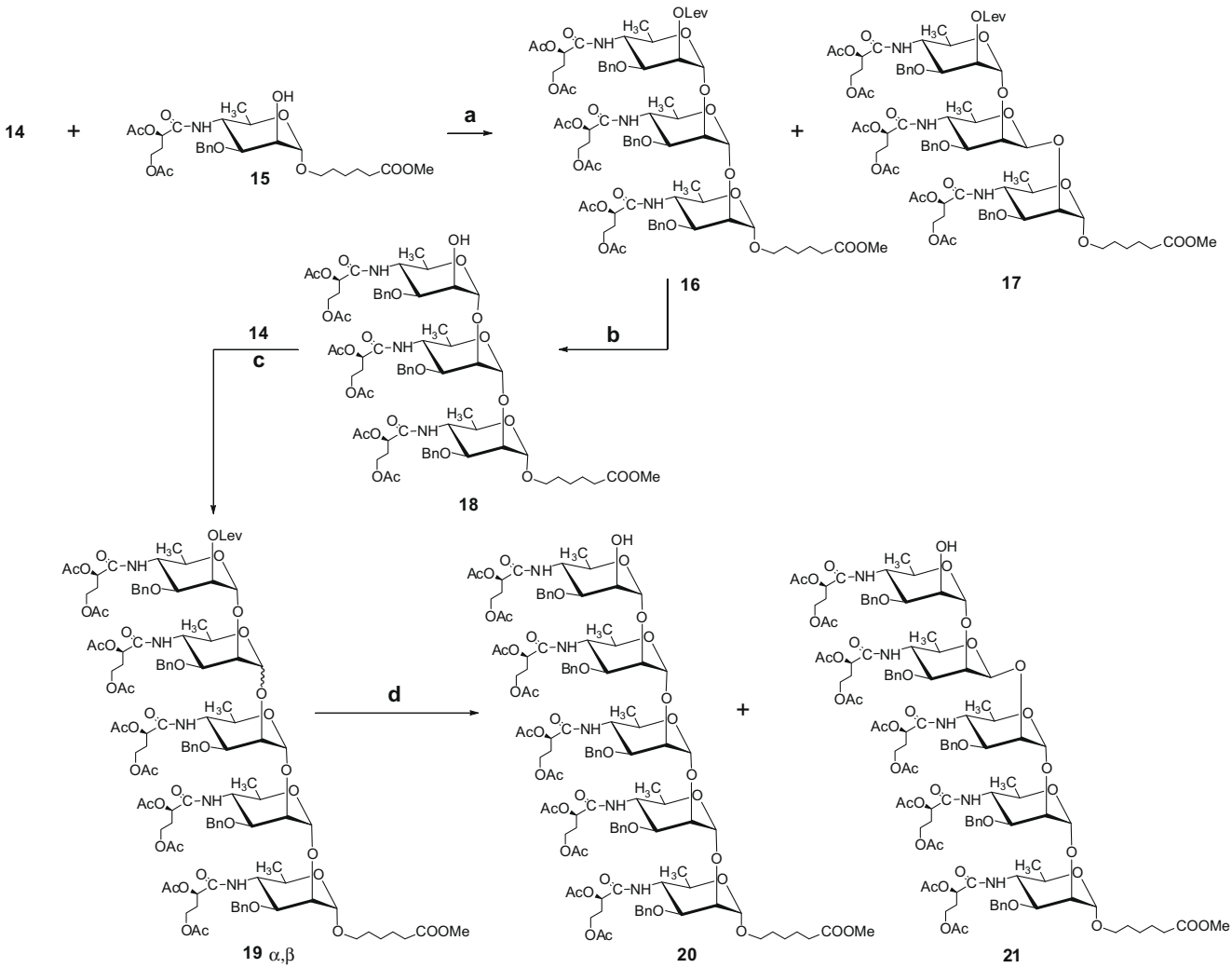
To compare the stereoselectivity of formation of an α-perosaminyl linkage from thioglycoside **3**[11] with that using the correspond-



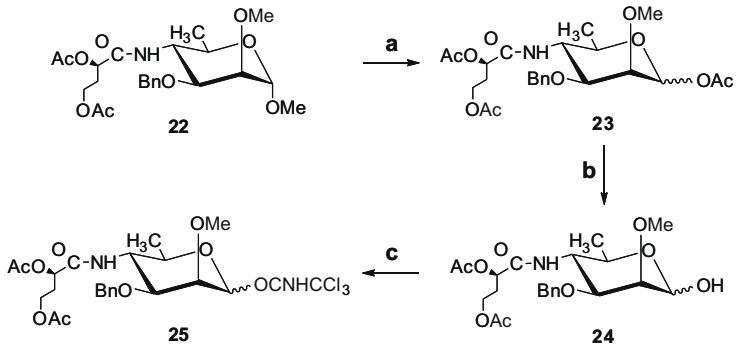
ing trichloroacetimidate, we synthesized imidate **14** (Scheme 1) and treated it with alcohols **15** and **18** (Scheme 2). When the reaction of **14** and **15** was carried out in DCM at −35 °C, a considerable amount of the β-linked trisaccharide **17** was formed (α:β ~2.5:1, NMR, unpublished results). The proclivity of this transformation to thermodynamic control became evident when the same synthons were allowed to react either at room temperature or at 100 °C, in toluene. Thus, while at room temperature the trisaccharides were formed in the α:β ratio of 10:1, the reaction in hot toluene led to compounds **16** and **17** in the ratio of 20:1. It should be



Scheme 1. Reagents and conditions: (a) piperidine, THF, rt, 95%; (b) DBU, Cl_3CCN, DCM, 0 °C, 71%; (c) hydrazine acetate, DCM–MeOH (10:1, v/v), rt, 86%; (d) **10**, TMSOTf, 4 Å MS, toluene–DCM (3:1, v/v), rt, 76%; (e) piperidine, THF, 0 °C to rt, 75%; (f) DBU, Cl_3CCN, DCM, 0 °C to rt, 84%.



Scheme 2. Reagents and conditions: (a) TMSOTf, 4 Å MS, toluene, 100 °C, 86%; (b) hydrazine acetate, DCM–MeOH (10:1, v/v), rt, 87%; (c) **14**, TMSOTf, 4 Å MS, toluene, 100 °C, 75%; (d) hydrazine acetate, DCM–MeOH (10:1, v/v), rt, 91%.



Scheme 3. Reagents and conditions: (a) Ac_2O–HOAc–H_2SO_4 (10:4:0.1, v/v), rt, 91%; (b) piperidine, THF, rt, 75%; (d) DBU, Cl_3CCN, DCM, 0 °C to rt, 86%.

noted that the glycosylation yields in these three reactions were high (86–95%). Reaction of **14** and alcohol **18** under thermodynamic control gave pentasaccharide **19** α,β with the same high stereoselectivity (α:β ratio, ~20:1, NMR). Separation of the two pentasaccharides was difficult and the mixture of anomers was resolved after removal of the levulinoyl group to give **20** and **21**.

Synthesis of the hexasaccharide fragments of the O-PS of *V. cholerae* O1, serotypes Inaba and Ogawa, required extension of pentasaccharide **20** at *O*-2 with glycosyl donor **10** and its 2-*O*-methyl analog **25**, respectively.

To obtain imidate **25**, the known[8] glycoside **22** was subjected sequentially to acetolysis (→**23**), anomeric deacetylation, and conversion of the free sugar **24** thus formed trichloroacetimidate **25**. We have previously observed[21] high stereoselectivity of formation of the α-perosaminyl linkage under conventional, not thermodynamically controlled conditions (room temperature, CH_2Cl_2 as solvent) using a different fully assembled monosaccharide glycosyl donor, namely thioglycoside **32**. Therefore, alcohol **20** was treated with each of imidates **10** and **25** (Scheme 4) under the same conditions. Both reactions were very slow, and some unchanged glycosyl donor was still present in both reaction mixtures after 16 h of reaction time. The β-linked hexasaccharides could not be found among the reaction products (NMR spectra of isolated, minor byproducts), indicating excellent stereoselectivity of formation of **26** and **27**, which were isolated in 73% and 69% yield, respectively. Hydrogenolysis of the foregoing fully protected intermediates gave alcohols **28** and **30**, respectively. Treatment of the former with NaOMe in MeOH effected simultaneous removal of protecting acetyl and levulinoyl groups to give the fully deprotected hexasaccharide **29**. Treatment of the Ogawa compound **30** with ethylenediamine effected simultaneous deacetylation of the side chains and amidation of the spacer to give the final hexasaccharide **31**, which is amenable for conjugation by squaric acid chemistry (Scheme 3).[22]

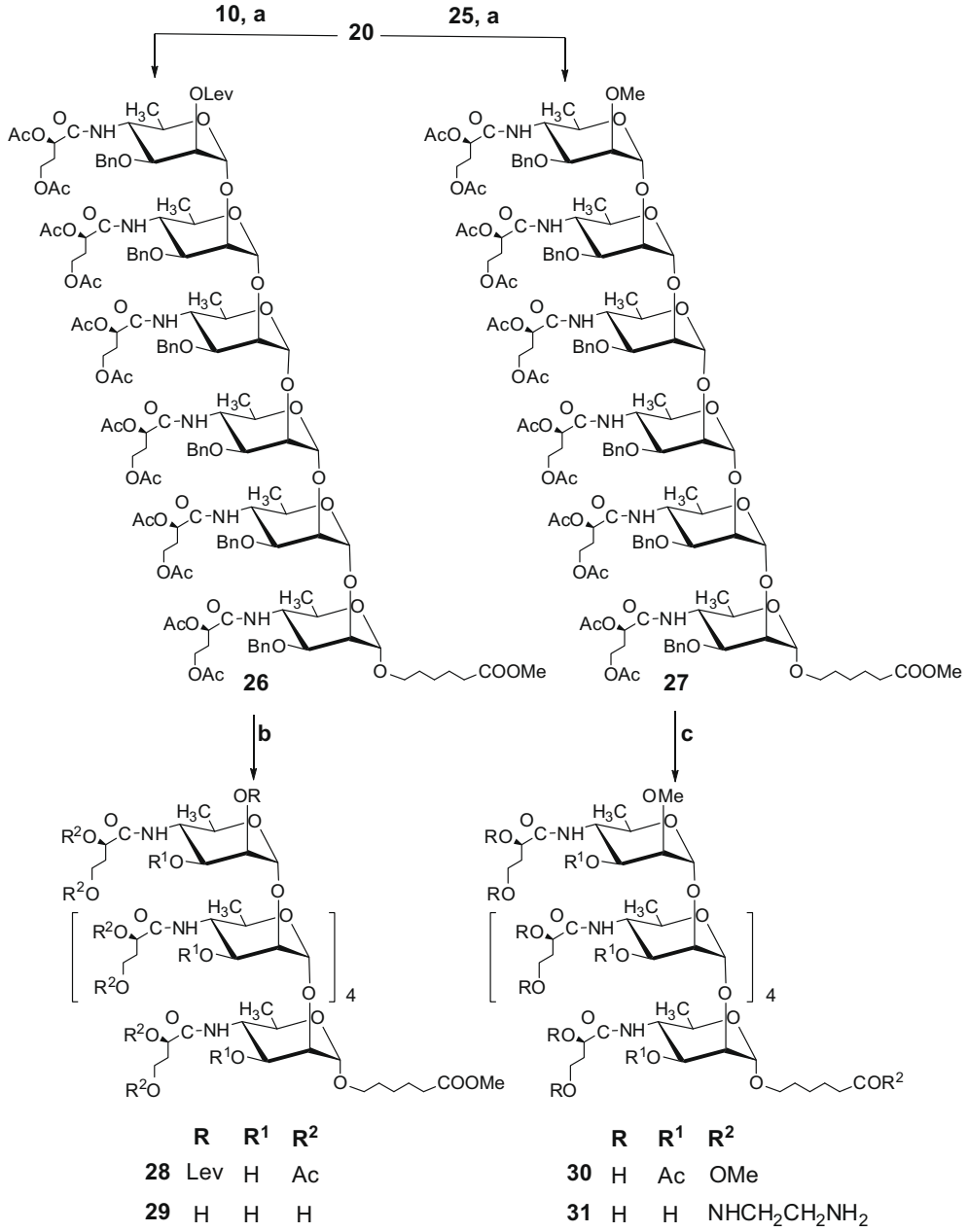
3. Conclusions

Results of this study confirm our previous[11] observation that the presence of the 3-deoxy-L-*glycero*-tetronamido side chain in glycosyl donors derived from perosamine significantly affects the stereoselectivity of glycosylation. When such glycosyl donors bear a *nonparticipating* group (*O*-alkyl or *O*-glycosyl) at the position vicinal to the active glycosidic center, the stereochemical outcome of perosaminylations—conducted by us[7,11,15,21,23] and elsewhere[16,17,24]—can be summarized as follows. Firstly, the formation of α-glycosidic linkage is highly favored when *mono*- and *oligo*saccharide glycosyl donors having azido group at O-4 are employed. Secondly, the α glycosidic linkage can also be stereoselectively formed from *mono*saccharide glycosyl donors where the 4-*N*-side chain is already in place (e.g., **2** or **32**), regardless of the *O*-2 substituent. Thirdly, and in contrast to their monosaccharide counterparts, the stereoselectivity of glycosylation conducted at conventional reaction conditions is poor when the glycosyl donor, a thioglycoside, glycosyl chloride, or trichloroacetimidate, is an *oligo*saccharide made from α-(1→2)-linked perosamine having the 4-(3-deoxy-L-*glycero*-tetronamido) side chain already in place. In such situations, the α stereoselectivity can be markedly improved by conducting the reaction under thermodynamic control. The benefit of the thermodynamic control is more pronounced when glycosylation is effected by the use of trichloroacetimidate, which are preferred for this purpose, to the corresponding thioglycosides or glycosyl chlorides.[11]

4. Experimental

4.1. General methods

Unless stated otherwise, optical rotations were measured at ambient temperature with a Perkin–Elmer automatic polarimeter, Model 341. All reactions were monitored by thin-layer chromatography (TLC) on Silica Gel 60 coated glass slides. Column chromatography was performed by elution from columns of silica gel with the CombiFlash Companion Chromatograph (Isco, Inc.) or Isolera Flash Chromatograph (Biotage). Solvent mixtures less polar than those used for TLC were used at the onset of separation. Nuclear magnetic resonance (NMR) spectra were measured at 300 MHz (^{1}H) and 75 MHz (^{13}C) with a Varian Gemini or Varian Mercury spectrometer, or at 600 MHz (^{1}H) and 150 MHz (^{13}C) with a Bruker Avance 600 spectrometer. Assignments of NMR signals were made by homonuclear and heteronuclear two-dimensional correlation spectroscopy, run with the software supplied with the spectrometers. Assignment of ^{13}C NMR spectra of some higher oligosaccharides was aided by comparison with spectra of related substances reported previously from this laboratory. 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.[25,26] 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 D-perosamine residues in the molecule. Nevertheless, often only incomplete assignment was achieved because of the overlap of resonances. When reporting assignment of NMR signals, nuclei associated with the 4-amido side chain are denoted with a prime and those with the spacer (linker) are denoted with a double prime. When reporting assignments of NMR signals, 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. Signals for sugar ring nuclei lacking Roman numeral assignment may belong to any of the rings.



Scheme 4. Reagents and conditions: (a) TMSOTf, 4 Å MS, DCM, rt, 73% for **27** and 69% for **28**; (b) (i) 5% Pd/C, H_2, DCM–MeOH (1:10, v/v), rt; (ii) NaOMe, MeOH, rt, 89% over two steps; (c) (i) 5% Pd/C, H_2, DCM–MeOH (1:10, v/v), rt, 87%; (ii) $H_2NCH_2CH_2NH_2$, 50 °C, 75%.

Only resonances that could be confidently assigned are listed in signal assignments. When reporting preparation of known substances, their identity was confirmed by comparison of NMR characteristics with those published previously. Liquid chromatography–electron spray-ionization mass spectrometry (ESI-MS) was performed with a Hewlett–Packard 1100 MSD spectrometer. Attempts have been made to obtain correct combustion analysis data for all new compounds. However, some compounds tenaciously retained traces of solvents, despite exhaustive drying, and analytical figures for carbon could not be obtained within 0.4%. Structures of these compounds follow unequivocally from the mode of synthesis, NMR spectroscopic data, and *m*/*z* values found in their mass spectra, and their purity was verified by TLC and NMR spectroscopy. Five percent palladium-on-charcoal catalyst (Escat|103) was purchased from Engelhard Industries. 1-(3-Dimethylaminopropyl)-3-ethyl-carbodiimide (EDAC) was purchased from ACROS Organics. Rubber septa used to close reaction flasks containing organic solvents were protected with a thin Teflon| sheet to avoid leaching. Solutions in organic solvents were dried with anhydrous Na_2SO_4 and concentrated at 40 °C/2 kPa.

4.2. 1-*O*-Acetyl-3-*O*-benzyl-4-(2,4-di-*O*-acetyl-3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-α-D-mannopyranose (11)

A solution of hydrazine acetate (4.10 g, 44.6 mmol) in MeOH (50 mL) was added to a stirred mixture of **8**[11] (18.4 g, 31.9 mmol) in DCM (500 mL). The stirring was continued until TLC (3:1 toluene–acetone) showed that the starting material was completely consumed (~6 h). After concentration, chromatography (3:1 hexane–EtOAc) afforded **11** (13.2 g, 86%), $[\alpha]_D$ +16 (*c* 0.5, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$) δ: 7.40–7.26 (m, 5H, Ph), 6.14 (d, $J_{1,2}$ = 1.2 Hz, H-1), 6.03 (d, *J* = 8.3 Hz, 1H, NH), 5.14 (dd, *J* = 8.0 Hz and 4.9 Hz, 1H, H-2′), 4.67–4.51 (AB_q, *J* = 12.0 Hz, 2H, PhCH_2), 4.18–3.99 (m, 6H, 2-OH, H-3, H-4, H-5, H-4′), 3.85 (m, 1H, H-2),

2.12–2.02 (m, 11H, $3COCH_3$, H-4′), 1.24 (d, $J_{5,6}$ = 6.4 Hz, 3H, H-6); ^{13}C NMR (150 MHz, $CDCl_3$) δ: 95.4 (C-1), 137.2 (C_q), 92.8 (C-1), 74.9 (C-3), 71.4 (PhCH_2), 71.1 (C-2′), 68.9 (C-5), 66.3 (C-4), 59.9 (C-4′), 52.7 (C-2), 30.9 (C-3′), 21.0, 20.9, 20.8 ($3CH_3CO$), 17.9 (C-6); TOF–MS *m*/*z*: 504 $[M+Na]^+$. Anal. Calcd for $C_{23}H_{31}NO_{10}$: C, 57.37; H, 6.49. Found: C, 57.11; H, 6.55.

4.3. 1-*O*-Acetyl-3-*O*-benzyl-4-(2,4-di-*O*-acetyl-3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-2-*O*-levulinoyl-α-D-mannopyranosyl-(1→2)-3-*O*-benzyl-4-(2,4-di-*O*-acetyl-3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-α-D-mannopyranose (12)

Piperidine (54 mL, 549 mmol) was added with stirring to a solution of **8**[27] (15.7 g, 27.1 mmol) in THF (250 mL) and the stirring was continued at room temperature until TLC (2:1 hexane–acetone) showed that the reaction was complete. The mixture was diluted with DCM (1000 mL) and washed successively with ice-cooled 0.5 M HCl (2 × 250 mL), satd $NaHCO_3$ (200 mL), and brine (200 mL), and the organic phase was dried and concentrated. Chromatography (2:1, hexane–acetone) gave 3-*O*-benzyl-4-(2,4-di-*O*-acetyl-3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-2-*O*-levulinoyl-α,β-D-mannopyranose (**9**, 13.8 g, 95%) as a mixture of anomers (α:β ~6:1). ^1H NMR (α product, 300 MHz, $CDCl_3$) δ: 7.35–7.26 (m, 5H, Ph), 5.82 (d, *J* = 7.9 Hz, 1H, NH), 5.40 (t, *J* = 2.06 Hz, 1H, H-2), 5.20–5.16 (m, 2H, H-1 and H-2′), 4.66–4.33 (AB_q, *J* = 12.0 Hz, 2H, PhCH_2), 4.16–3.90 (m, 5H, H-3, H-4, H-5, H-4′), 2.76–2.64 (m, 4H, CH_2CH_2), 2.20–2.00 (m, 11H, $3COCH_3$ and H-3′), 1.21 (d, $J_{5,6}$ = 6.0 Hz, H-6); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 172.2 (CO), 169.8 (CO), 169.6 (CO), 137.9 (C_q), 92.5 (C-1), 73.2 (C-3), 71.3 (C-2′), 70.8 (PhCH_2), 67.8 (C-2 and C-5), 60.2 (C-4′), 53.0 (C-4), 38.2 (CH_2CO), 31.1 (C-3′), 30.0 ($OCOCH_2$), 28.3 ($COCH_3$), 21.0 ($2CH_3CO$), 18.3 (C-6); TOF-HRMS *m*/*z*: $[M+H]^+$ calcd for $C_{26}H_{36}NO_{11}$: 538.2288. Found 538.2278.

A solution of compound **9** (600 mg, 1.19 mmol) in DCM (10 mL) was treated at 0 °C with Cl_3CCN (1.2 mL, 12 mmol) in the presence of DBU (0.09 mL, 0.6 mmol) for 2 h and concentrated. Chromatography (3:2 hexane–EtOAc) gave 3-*O*-benzyl-4-(2,4-di-*O*-acetyl-3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-2-*O*-levulinoyl-α-D-mannopyranose 1-*O*-trichloroacetimidate (**10**, 540 mg, 71%). ^1H NMR (300 MHz, $CDCl_3$) δ: 8.71 (s, 1H, NH), 7.35–7.28 (m, 5H, Ph), 6.23 (d, $J_{1,2}$ = 1.92 Hz, H-1), 5.80 (d, *J* = 7.4 Hz, 1H, NH), 5.51 (t, *J* = 2.2 Hz, 1H, H-2), 5.17 (dd, *J* = 7.9 Hz and 4.8 Hz, 1H, H-2′), 4.66–4.37 (AB_q, *J* = 12.0 Hz, 2H, PhCH_2), 4.15–3.93 (m, 5H, H-3, H-4, H-5, H-4′), 2.79–2.71 (m, 4H, CH_2CH_2), 2.18–2.05 (m, 11H, $3COCH_3$ and H-3′), 1.25 (d, $J_{5,6}$ = 5.6 Hz, H-6); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 95.4 (C-1), 72.5 (C-3), 71.3 (C-2′), 71.09, 70.6 (PhCH_2), 66.25 (C-2 and C-5), 60.1 (C-4′), 52.7 (C-4), 38.1 (CH_2CO), 31.1 (C-3′), 30.0 ($OCOCH_2$), 28.2 ($COCH_3$), 21.0 ($2CH_3CO$), 18.3 (C-6); TOF-HRMS *m*/*z*: $[M+H]^+$ calcd for $C_{28}H_{36}N_2O_{11}Cl_3$: 681.1385. Found 681.1388.

A mixture of **10** (9.8 g, 14.4 mmol), **11** (6.29 g, 13.0 mmol), and 4 Å MS (2.1 g) in toluene–DCM (3:1, v/v 150 mL) was stirred under N_2 at room temperature for 30 min. TMSOTf (16 μl, 0.65 mmol) was added, and the stirring was continued for 4 h when TLC (3:2 hexane–acetone) showed that the donor was completely consumed. After addition of Et_3N (1.0 mL), the mixture was filtered through Celite pad, the filtrate was concentrated, and chromatography (3:1→3:2 hexane–acetone) gave **12** (10.0 g, 76%). $[\alpha]_D$ −120 (*c* 0.92, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$) δ: 7.30–7.17 (m, 10H, 2Ph), 6.02 (d, *J* = 8.7 Hz, 1H, NH^I), 5.99 (d, $J_{1,2}$ = 2.2 Hz, 1H, $H\text{-}1^I$), 5.68 (d, *J* = 9.0 Hz, 1H, NH^{II}), 5.41 (t, *J* = 2.5 Hz, $H\text{-}2^{II}$), 5.09 (m, 2H, $H\text{-}2'^{I,II}$), 4.85 (d, $J_{1,2}$ = 1.8 Hz, $H\text{-}1^{II}$), 4.60–4.30 (m, 4H, 2PhCH_2), 4.04–3.95 (m, 7H, $H\text{-}3^I$, $H\text{-}5^I$, $H\text{-}4^{II}$, $H\text{-}4'^{I,II}$), 3.85 (t, *J* = 2.5 Hz, 1H, $H\text{-}2^I$), 3.71–3.67 (m, 3H, $H\text{-}4^I$, 3^{II}, $H\text{-}5^{II}$), 2.62 (m, 4H, CH_2CH_2), 2.10–1.92 (m, 22H, $6COCH_3$, $H\text{-}3'^{I,II}$), 1.15 (m, 6H, $H\text{-}6^{I,II}$); ^{13}C NMR (150 MHz, $CDCl_3$) δ: 137.6 (C_q), 137.5 (C_q), 99.4 ($C\text{-}1^{II}$), 92.5 ($C\text{-}1^I$), 74.2 ($C\text{-}3^I$), 73.3 ($C\text{-}3^{II}$), 72.6 ($C\text{-}2^I$), 71.9 (PhCH_2), 71.3 and 71.2 ($C\text{-}2'^{I,II}$), 70.7 (PhCH_2), 69.6 ($C\text{-}5^{II}$), 69.0 ($C\text{-}5^I$), 67.2 ($C\text{-}2^{II}$), 60.1 and 60.0 ($C\text{-}4'^{I,II}$), 53.4 ($C\text{-}4^I$), 51.9 ($C\text{-}4^{II}$), 38.0 (CH_2CO), 31.0 and 30.9 ($C\text{-}3'^{I,II}$), 29.8 ($COCH_3$), 28.2 ($OCOCH_2$), 21.0, 20.9, 20.8 (5C, $5COCH_3$), 18.1 and 17.9 ($C\text{-}6^{I,II}$); TOF-HRMS *m*/*z*: $[M+H]^+$ calcd for $C_{49}H_{65}N_2O_{20}$: 1001.4131. Found 1001.4139. Anal. Calcd for $C_{49}H_{64}N_2O_{20}$: C, 58.79; H, 6.44. Found: C, 58.60; H, 6.48.

4.4. 5-(Methoxycarbonyl)pentyl 3-*O*-benzyl-4-(2,4-di-*O*-acetyl-3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-2-*O*-levulinoyl-α-D-mannopyranosyl-(1→2)-3-*O*-benzyl-4-(2,4-di-*O*-acetyl-3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-α-D-mannopyranosyl-(1→2)-3-*O*-benzyl-4-(2,4-di-*O*-acetyl-3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-α-D-mannopyranoside (16)

Piperidine (3.6 mL, 36 mmol) was added with stirring at 0 °C to a solution of **12** (1.32 g, 1.3 mmol) in THF (20 mL) and the mixture was allowed to warm up to room temperature. After 24 h, the mixture was diluted with DCM (200 mL) and washed with 0.5 M HCl (50 mL) and saturated $NaHCO_3$ aq (50 mL), dried, concentrated, and chromatography (3:1→95:5 EtOAc–hexane) gave 3-*O*-benzyl-4-(2,4-di-*O*-acetyl-3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-2-*O*-levulinoyl-α-D-mannopyranosyl-(1→2)-3-*O*-benzyl-4-(2,4-di-*O*-acetyl-3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-α-D-mannopyranose (**13**, 940 mg, 75%). ^1H NMR (600 MHz, $CDCl_3$) δ: 7.36–7.27 (m, 10H, 2Ph), 5.91 (d, *J* = 8.3 Hz, 1H, NH^I), 5.82 (d, *J* = 9.2 Hz, 1H, NH^{II}), 5.49 (t, *J* = 2.5 Hz, $H\text{-}2^{II}$), 5.20–5.15 (m, 3H, $H\text{-}1^I$, $H\text{-}2'^{I,II}$), 4.87 (d, $J_{1,2}$ = 1.9 Hz, $H\text{-}1^{II}$), 4.65–4.38 (m, 4H, 2PhCH_2), 4.20–3.90 (m, 9H, $H\text{-}2^I$, $H\text{-}3^I$, $H\text{-}4^I$, $H\text{-}5^I$, $H\text{-}4^{II}$, $H\text{-}4'^{I,II}$), 3.81–3.74 (m, 2H, 3^{II}, $H\text{-}5^{II}$), 3.10 (br s, 1H, 1-OH), 2.76–2.60 (m, 4H, CH_2CH_2), 2.10–1.92 (m, 19H, $5COCH_3$, $H\text{-}3'^{I,II}$), 1.18 (d, $J_{5,6}$ = 5.8 Hz, 3H, $H\text{-}6^I$), 1.15 (d, $J_{5,6}$ = 6.3 Hz, 3H, $H\text{-}6^{II}$); ^{13}C NMR (150 MHz, $CDCl_3$) δ: 137.8 (C_q), 137.6 (C_q), 99.5 ($C\text{-}1^{II}$), 93.4 ($C\text{-}1^I$), 74.5 ($C\text{-}2^I$, $C\text{-}3^I$), 72.9 ($C\text{-}3^{II}$), 71.3 (PhCH_2), 71.2 and 71.0 ($C\text{-}2'^{I,II}$), 70.4 (PhCH_2), 68.5 ($C\text{-}5^{II}$), 67.9 ($C\text{-}5^I$), 67.1 ($C\text{-}2^{II}$), 60.0 ($C\text{-}4'^{I,II}$), 52.6 ($C\text{-}4^I$), 51.9 ($C\text{-}4^{II}$), 38.0 (CH_2CO), 31.0 and 30.9 ($C\text{-}3'^{I,II}$), 29.8 ($COCH_3$), 28.2 ($OCOCH_2$), 20.9 ($2COCH_3$), 20.8 ($2COCH_3$), 18.1 and 17.9 ($C\text{-}6^{I,II}$); TOF-HRMS *m*/*z*: $[M+H]^+$ calcd for $C_{47}H_{63}N_2O_{19}$: 959.4052. Found 959.4014.

DBU (0.18 mL, 0.9 mmol) was added with stirring at 0 °C to a mixture of **13** (9.0 g, 9.4 mmol), $CNCCl_3$ (4.7 mL, 47.0 mmol), and DCM (150 mL), the mixture was allowed to warm up to room temperature and stirred overnight. After concentration, chromatography (3:1→3:2 hexane–acetone) gave 3-*O*-benzyl-4-(2,4-di-*O*-acetyl-3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-2-*O*-levulinoyl-α-D-mannopyranosyl-(1→2)-3-*O*-benzyl-4-(2,4-di-*O*-acetyl-3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-α-D-mannopyranose-1-*O*-trichloroacetimidate (**14**, 8.7 g, 84%). ^1H NMR (600 MHz, $CDCl_3$) δ: 8.62 (s, 1H, $OCNHCCl_3$), 7.37–7.27 (m, 10H, 2Ph), 6.15 (d, $J_{1,2}$ = 2.0 Hz, $H\text{-}1^I$), 5.89 (d, *J* = 7.8 Hz, 1H, NH^I), 5.74 (d, *J* = 9.0 Hz, 1H, NH^{II}), 5.51 (br t, *J* = ~2.4 Hz, $H\text{-}2^{II}$), 5.23–5.17 (m, 2H, $H\text{-}2'^{I,II}$), 4.91 (d, $J_{1,2}$ = 1.7 Hz, $H\text{-}1^{II}$), 4.68–4.40 (m, 4H, 2PhCH_2), 4.20–3.97 (m, 9H, $H\text{-}4'I\text{-}^{II}$, $H\text{-}4^{II}$, $H\text{-}2^I$, $H\text{-}5^I$, $H\text{-}3^I$, $H\text{-}4^I$), 3.78–3.76 (m, 2H, 3^{II}, $H\text{-}5^{II}$), 2.75–2.65 (m, 4H, CH_2CH_2), 2.10–1.92 (m, 19H, $5COCH_3$, $H\text{-}3'^{I,II}$), 1.24 (d, $J_{5,6}$ = 6.3 Hz, 3H, $H\text{-}6^I$), 1.19 (d, $J_{5,6}$ = 6.3 Hz, 3H, $H\text{-}6^{II}$); ^{13}C NMR (150 MHz, $CDCl_3$) δ: 137.7 (C_q), 137.4 (C_q), 99.8 ($C\text{-}1^{II}$), 96.5 ($C\text{-}1^I$), 74.2 ($C\text{-}3^I$), 73.1 ($C\text{-}3^{II}$), 72.6 ($C\text{-}2^I$), 71.9 (PhCH_2), 71.3 and 71.2 ($C\text{-}2'^{I,II}$), 70.8 ($C\text{-}5^I$), 70.7 (PhCH_2), 69.1 ($C\text{-}5^{II}$), 67.2 ($C\text{-}2^{II}$), 60.1 ($C\text{-}4'^{I,II}$), 52.7 ($C\text{-}4^{II}$), 51.9 ($C\text{-}4^I$), 38.2 (CH_2CO), 31.2 and 31.0 ($C\text{-}3'^{I,II}$), 30.0 ($COCH_3$), 28.3 ($OCOCH_2$), 21.1 ($2COCH_3$), 21.0 ($2COCH_3$), 18.3 and 18.0 ($C\text{-}6^{I,II}$); TOF–MS *m*/*z*: 1127 $[M+Na]^+$.

A solution of TMSOTf in toluene (0.03 M, 10 mL) was added at 100 °C under N_2 to a stirred mixture of **14** (7.5 g, 6.79 mmol), 5-(methoxycarbonyl)pentyl 3-*O*-benzyl-4-(2,4-di-*O*-acetyl-3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-α-D-mannopyranoside (**15**,[11]

3.5 g, 6.17 mmol), 4 Å molecular sieves (10 g), and toluene (300 mL). After 5 h, when TLC (3:2 hexane–acetone) showed that donor **14** was consumed, the mixture was cooled to room temperature and Et_3N (0.8 mL) was added. The mixture was filtered through a Celite pad, the filtrate was concentrated, and chromatography (3:1→3:2 hexane–acetone) gave the unchanged glycosyl acceptor **15** (0.83 g) and a mixture of known[8] α-linked compound **16** and 5-(methoxycarbonyl)pentyl 3-*O*-benzyl-4-(2,4-di-*O*-acetyl-3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-2-*O*-levulinoyl-α-D-mannopyranosyl-(1→2)-3-*O*-benzyl-4-(2,4-di-*O*-acetyl-3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-β-D-mannopyranosyl-(1→2)-3-*O*-benzyl-4-(2,4-di-*O*-acetyl-3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-α-D-mannopyranoside (**17**) (combined yield, 7.8 g, glycosylation yield 84%, **16**:**17** ~20:1). The mixture was rechromatographed (12:1 toluene–2-propanol) to give **16** (6.22 g) and **17** (0.21 g). A small amount of unresolved mixture of **16** and **17** was also obtained.

Compound **17**: ^{1}H NMR (600 MHz, $CDCl_3$) δ: 7.92 (d, J = 9.0 Hz, 1H, NH^{III}), 7.36–7.08 (m, 15H, 3Ph), 6.43 (d, J = 10.0 Hz, 1H, NH^{I}), 6.25 (d, J = 7.4 Hz, 1H, NH^{II}), 5.47 (d, $J_{1,2}$ = 1.3 Hz, 1H, H-1^{III}), 5.44 (dd, $J_{1,2}$ = 1.3 Hz, $J_{2,3}$ = 3.1 Hz, 1H, H-2^{III}), 5.26 (dd, J = 5.4 Hz, J = 7.6 Hz, 1H, H-2′), 5.02 (m, 2H, H-2′), 4.80 (d, J = 10.3 Hz, 1H, PhCH_2), 4.75 (d, $J_{1,2}$ = 1.6 Hz, 1H, H-1^{I}), 4.67 (d, J = 11.3 Hz, 1H, PhCH_2), 4.56 (s, 1H, H-1^{II}), 4.36 (d, $J_{2,3}$ = 2.2 Hz, H-2^{II}), 4.28 (d, J = 11.3 Hz, 1H, PhCH_2), 4.24–4.21 (m, 2H, H-2^{I}, H-4^{III}), 4.10–3.98 (m, 13H, PhCH_2, H-4^{I}, H-3^{II}, H-5^{II}, H-5^{III}, H-$4'^{I,II,III}$), 3.77 (dd, $J_{2,3}$ = 3.1 Hz, $J_{3,4}$ = 10.9 Hz, H-3^{III}), 3.64–3.61 (m, 6H, OCH_3, H-1″$_a$, H-3^{I} and H-5^{I}), 3.37–3.34 (m, 2H, H-4^{II}, H-1″$_b$), 2.56–2.51 (m, 4H, CH_2CH_2), 2.29 (t, J = 7.5 Hz, 2H, H-5″$_{a,b}$), 2.02–1.97 (m, 27H, 5$COCH_3$, H-$3'^{I,II,III}$), 1.62–1.54 (m, 4H, H-2″$_{a,b}$, H-4″$_{a,b}$), 1.28 (m, 2H, H-3″$_{a,b}$), 1.26 (d, $J_{5,6}$ = 6.3 Hz, 3H, H-6^{I}), 1.19 (d, $J_{5,6}$ = 6.3 Hz, 3H, H-6^{II}); ^{13}C NMR (150 MHz, $CDCl_3$) δ: 97.4 (2C, C-1^{I}, C-1^{II}), 96.9 (C-1^{III}), 77.3 (C-3^{III}), 74.2 (C-3^{I}), 71.7 (C-2′), 71.6 (PhCH_2), 71.5 (PhCH_2), 71.1 (C-2′), 70.8 (C-2′), 69.6 (C-2^{I}, C-5^{III}), 69.4 (PhCH_2), 69.3 (C-5^{II}), 68.8 (C-2^{III}), 67.2 (C-5^{I}), 76.1 (C-1″), 66.9 (C-2^{II}), 60.9 (C-4′), 60.1 (C-4′), 59.9 (C-4′), 55.7 (C-4^{II}), 51.8 (C-4^{I}, C-4^{II}), 38.4 (2C, CH_2CH_2), 34.0 (C-5″), 31.3(C-3′), 31.2 (C-3′), 30.6 (C-3′), 28.8 (C-2″), 25.6 (C-3″), 24.4 (C-4″), 21.0 (2COCH_3), 20.9 (2COCH_3), 20.8 (2COCH_3), 18.4, 18.1 and 18.0 (C-$6^{I,II,III}$); TOF-HRMS m/z: $[M+Na]^+$ calcd for $C_{75}H_{101}N_3O_{29}Na$: 1530.6418. Found 1530.6324.

4.5. 5-(Methoxycarbonyl)pentyl 3-*O*-benzyl-4-(2,4-di-*O*-acetyl-3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-α-D-mannopyranosyl-(1→2)-3-*O*-benzyl-4-(2,4-di-*O*-acetyl-3-deoxy-L-*glycero*tetronamido)-4,6-dideoxy-α-D-mannopyranosyl-(1→2)-3-*O*-benzyl-4-(2,4-di-*O*-acetyl-3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-α-D-mannopyranoside (18)

Compound **16** (6.2 g, 4.1 mmol) was treated with hydrazine acetate, as described for preparation of **11**, to give trisaccharide **18** (5.0 g, 87%), which was identical (TLC, NMR) with the known, independently synthesized[11] substance.

4.6. 5-(Methoxycarbonyl)pentyl 3-*O*-benzyl-4-(2,4-di-*O*-acetyl-3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-α-D-mannopyranosyl-(1→2)-3-*O*-benzyl-4-(2,4-di-*O*-acetyl-3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-α-D-mannopyranosyl-(1→2)-3-*O*-benzyl-4-(2,4-di-*O*-acetyl-3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-α-D-mannopyranosyl-(1→2)-3-*O*-benzyl-4-(2,4-di-*O*-acetyl-3-deoxy-L-*glycero*-tetronamido)4,6-dideoxy-α-D-mannopyranosyl-(1→2)-3-*O*-benzyl-4-(2,4-di-*O*-acetyl-3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-α-D-mannopyranoside (20)

A solution of TMSOTf in toluene (0.03 M, 6 mL) was added with stirring at 100 °C under N_2 to a mixture of **18**[11] (5.0 g, 3.5 mmol), **14** (4.69 g, 4.25 mmol), and 4 Å molecular sieves (5.8 g) in toluene (110 mL). After 3 h, when TLC (1:1 hexane–acetone) showed that the reaction was virtually complete, the mixture was cooled to room temperature and Et_3N (0.8 mL) was added. The mixture was filtered through Celite pad, the filtrate was concentrated, and chromatography (6:1→3:1 hexane–acetone) gave an anomeric mixture of fully protected pentasaccharides **19α**,**β** (6.0 g, total yield 75%). TOF–MS m/z: 2374 $[M+Na]^+$. The foregoing anomeric mixture (6.0 g, 2.5 mmol) was treated with hydrazine acetate (202 mg, 3.0 mmol) in MeOH (400 mL) overnight. Work-up, as described above for a similar reaction, and chromatography (5:1 DCM–acetone), gave first the β-linked product, 5-(methoxycarbonyl)pentyl 3-*O*-benzyl-4-(2,4-di-*O*-acetyl-3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-α-D-mannopyranosyl-(1→2)-3-*O*-benzyl-4-(2,4-di-*O*-acetyl-3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-α-D-mannopyranosyl-(1→2)-3-*O*-benzyl-4-(2,4-di-*O*-acetyl-3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-β-D-mannopyranosyl-(1→2)-3-*O*-benzyl-4-(2,4-di-*O*-acetyl-3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-α-D-mannopyranosyl-(1→2)-3-*O*-benzyl-4-(2,4-di-*O*-acetyl-3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-α-D-mannopyranoside (**21**, 220 mg, 4%). ^{1}H NMR (600 MHz, $CDCl_3$) δ: 7.74 (br s, 1H, NH), 7.37–7.21 (m, 25H, 5Ph), 6.40–6.12 (m, 4H, 4NH), 5.45 (br s, 1H, H-1), 5.28 (dd, J = 4.8, 8.2 Hz, 1H, H-2′), 5.18–5.12 (m, 4H, 4 × H-2′), 5.04 (br s, 1H, H-1), 4.90 (br s, 1H, H-1), 4.78 (d, overlapped with CHPh, H-1), ~4.30 (m, H-1^{IV}, overlapped), 2.30 (t, J = 7.2 Hz, H-5″$_{a,b}$), 2.17–1.92 (m, 40H, H-$3'^{I-V}$, 10$COCH_3$), 1.67–1.63 (m, 4H, H-2″$_{a,b}$, H-4″$_{a,b}$), 1.38 (m, 2H, H-3″$_{a,b}$), 1.26–1.08 (m, 15H, H-6^{I-V}); ^{13}C NMR (150 MHz, $CDCl_3$) δ: 100.9 (C-1, $J_{C\text{-}1,H\text{-}1}$ 172 Hz), 99.6 (br, C-1, $J_{C\text{-}1,H\text{-}1}$ not determined), 98.8(C-1, $J_{C\text{-}1,H\text{-}1}$ 169.6 Hz), 98.6 (C-1, $J_{C\text{-}1,H\text{-}1}$ 172 Hz), 98.1(C-1, $J_{C\text{-}1,H\text{-}1}$ 154 Hz), 20.9 and 20.8 (5C, C-6^{I-V}); TOF–MS m/z: 2275 $[M+Na]^+$.

Eluted next was the all-α-linked pentasaccharide **20** (5.0 g, 87%), $[\alpha]_D$ −9.2 (c 1.08, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ: 7.40–7.24 (m, 25H, 5Ph), 6.53–6.13 (m, 4H, 4NH), 5.18 (m, 5H, H-$2'^{I-V}$), 5.08 (d, $J_{1,2}$ = 2.0 Hz, H-1), 4.09 (br s, 2H, 2H-1), 4.87 (br s, 1H, H-1), 4.71 (br s, 1H, H-1), 4.64–4.58 (m, 10H, 5PhCH_2), 4.20–4.08 (m, 20H, H-2^{I-V}, H-4^{I-V}, H-$4'^{I-V}$), 3.86–3.32 (m, 15H, H-3^{I-V}, H-5^{I-V}, OCH_3, H-1″$_{a,b}$), 2.46 (br s, 1H, OH-2^{V}), 2.32 (t, J = 7.2 Hz, H-5″$_{a,b}$), 2.21–1.95 (m, 40H, H-$3'^{I-V}$, 10$COCH_3$), 1.66–1.53 (m, 4H, H-2″$_{a,b}$, H-4″$_{a,b}$), 1.44–1.32 (m, 2H, H-3″$_{a,b}$), 1.14–1.08 (m, 15H, H-6^{I-V}). ^{13}C NMR (150 MHz, $CDCl_3$) δ: 100.7 (C-1), 99.9 (3C, 3C-1), 98.8 (C-1), 18.4 (C-6), 18.3 (C-6), 18.2 (2C, 2C-6), 17.9 (C-6). TOF-HRMS m/z: $[M+H]^+$ calcd for $C_{112}H_{150}N_5O_{43}$: 2252.9705. Found 2252.9724. Anal. Calcd for $C_{112}H_{149}N_5O_{43}$: C, 59.70; H, 6.66; N, 3.11. Found: C, 59.41; H, 6.70; N, 3.08.

4.7. 1-*O*-Acetyl-3-*O*-benzyl-4-(2,4-di-*O*-acetyl-3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-2-*O*-methyl-α-D-mannopyranose (23)

Methyl 3-*O*-benzyl-4-(2,4-di-*O*-acetyl-3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-2-*O*-methyl-α-D-mannopyranose (**22**,[8] 7.0 g, 15 mmol) was treated at room temperature with Ac_2O–HOAc–H_2SO_4 (10–4–0.1, 140 mL) for 1.5 h, and NaOAc·$3H_2O$ (2.0 g) was added to terminate the reaction. After concentration, chromatography (1:1 hexane–EtOAc) gave **23** (6.7 g, 91%), mp 104–105 °C (EtOH); $[\alpha]_D$ −2 (*c* 1.1, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ: 7.36–7.27 (m, 5H, Ph), 6.13 (d, $J_{1,2}$ = 1.9 Hz, 1H, H-1), 6.08 (d, J = 8.1 Hz, 1H, NH), 5.16 (dd, J = 8.0 Hz and 4.7 Hz, 1H, H-2′), 4.65–4.51 (AB_q, J = 11.7 Hz, 2H, PhCH_2), 4.18–4.07 (m, 4H, H-3, H-5 and H-4′), 3.80 (m, 1H, H-4), 3.50 (br s, 4H, H-2 and OCH_3), 2.19 (m, 1H, H-3_a′), 2.11–2.04 (m, 10H, 3CH_3CO and H-3_b′), 1.22 (d, $J_{5,6}$ = 6.3 Hz, 3H, H-6). ^{13}C NMR (150 MHz, $CDCl_3$) δ: 91.5 (C-1), 75.7 (C-2), 74.4 (C-3), 71.6 (PhCH_2), 71.3 (C-2′), 69.5 (C-5), 60.1 (C-4′), 59.3 (OCH_3), 53.8 (C-4), 31.1 (C-3′), 21.2, 21.0, 20.9 (3COCH_3), 18.6 (C-6); TOF-HRMS m/z: $[M+H]^+$ calcd for $C_{24}H_{34}NO_{10}$: 496.2183. Found 496.2178. Anal. Calcd for

$C_{24}H_{33}NO_{10}$: C, 58.17; H, 6.71; N, 2.83. Found: C, 58.36; H, 6.76; N 2.78.

4.8. (2-Aminoethylamido)carbonylpentyl 4-(3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-α-D-mannopyranosyl-(1→2)-tetrakis[-4-(3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-α-D-mannopyranosyl]-4-(3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-2-*O*-methyl-α-D-mannopyranoside (31)

Piperidine (4.0 mL, 40 mL) was added dropwise to a solution of **23** (1.0 g, 2.0 mmol) in THF (10 mL). After 3 h, when TLC (3:1 DCM–acetone) showed that the reaction was complete, the mixture was concentrated and chromatography (10:1 DCM–acetone) gave the intermediate **24**, 3-*O*-Benzyl-4-(2,4-di-*O*-acetyl-3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-2-*O*-methyl-α,β-D-mannopyranose (800 mg, 75%). TOF–MS: *m*/*z*: 476.1 $[M+Na]^+$.

DBU (0.08 mL, 0.5 mmol) was added with stirring at 0° C to a solution of **24** (2.2 g, 4.9 mmol) and CCl_3CN (2.5 mL, 24 mmol) in DCM (30 mL), and the mixture was allowed to warm up to room temperature. After 3 h, the mixture was concentrated and chromatography (3:1, hexane–acetone with 1% Et_3N) afforded 3-*O*-benzyl-4-(2,4-di-*O*-acetyl-3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-2-*O*-methyl-α-D-mannopyranose-1-*O*-trichloroacetimidate (**25**, 2.5 g, 86%). ^{1}H NMR (600 MHz, $CDCl_3$) δ: 8.58 (s, 1H, $NHCCl_3$), 7.36–7.27 (m, 5H, Ph), 6.27 (d, $J_{1,2}$ = 2.0 Hz, 1H, H-1), 5.95 (d, J = 8.3 Hz, 1H, NH), 5.16 (dd, J = 8.0 Hz and 4.7 Hz, 1H, H-2′), 4.64–4.54 (AB_q, J = 12.0 Hz, 2H, $PhCH_2$), 4.16–4.06 (m, 4H, H-3, H-5 and H-4′), 3.93 (m, 1H, H-4), 3.63 (t, J = 2.5 Hz, 1H, H-2), 3.53 (s, 3H, OCH_3), 2.18–2.03 (m, 8H, $2COCH_3$ and H-3′), 1.23 (d, $J_{5,6}$ = 6.4 Hz, 3H, H-6); ^{13}C NMR (150 MHz, $CDCl_3$) δ: 95.3 (C-1), 74.9 (C-2), 74.1 (C-3), 71.5 ($PhCH_2$), 71.0 (C-2′), 70.2 (C-5), 59.2 (C-4′), 59.2 (OCH_3), 52.9 (C-4), 30.8 (C-3′), 20.8 20.7 ($2COCH_3$), 18.0 (C-6); TOF-HRMS, *m*/*z*: calcd for $C_{24}H_{31}N_2O_9NaCl_3$ $[M+Na]^+$: 619.0993. Found 619.0969.

A solution of TMSOTf in toluene (0.03 M, 11 mL) was added with exclusion of moisture to a stirred mixture of **25** (1.6 g, 2.68 mmol), **20** (4.0 g, 1.77 mmol), 4 Å molecular sieves (6.5 g) in DCM (100 mL), and the stirring was continued overnight, when TLC (3:1 DCM–acetone) showed that only small amount of the imidate **25** was present. The mixture was neutralized with Et_3N (1.0 mL), filtered, the filtrate was concentrated, and chromatography (5:1 DCM–acetone) gave the known[11] hexasaccharide, 5-(methoxycarbonyl)pentyl 3-*O*-benzyl-4-(2,4-di-*O*-acetyl-3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-α-D-mannopyranosyl-(1→2)-tetrakis[-3-*O*-benzyl-4-(2,4-di-*O*-acetyl-3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-α-D-mannopyranosyl]-3-*O*-benzyl-4-(2,4-di-*O*-acetyl-3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-2-*O*-methyl-α-D-mannopyranoside **27** (3.3 g, yield 69%).

A stirred solution of **27** (3.3 g) in DCM–MeOH (1: 10, 70 mL) was treated overnight with hydrogen in the presence of 5% Pd/C (2.0 g). After filtration and concentration, the residue was chromatographed (12:1 EtOAc–EtOH) to afford 5-(methoxycarbonyl)pentyl-4-(2,4-di-*O*-acetyl-3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-α-D-mannopyranosyl-(1→2)-tetrakis[-4-(2,4-di-*O*-acetyl-3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-α-D-mannopyranosyl]-4-(2,4-di-*O*-acetyl-3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-2-*O*-methyl-α-D-mannopyranoside **30** (2.25 g, 87%). TOF–MS *m*/*z*: 1074 $[M]^{2+}$, 2148 $[M]^+$.

The foregoing hexasaccharide **30** (2.23 g, 1.03 mmol) was treated with ethylenediamine (17 mL) at 50 °C overnight. After concentration, chromatography (1:1:0.1 MeOH–DCM–25% NH_4OH) afforded the known[20] **31** (1.3 g, 75%). ^{1}H NMR (400 MHz, D_2O) δ: 5.12–4.79 (m, 6H, H-1^{I-VI}), 4.21–4.18 (m, 6H, H-$2'^{I-VI}$), 4.08–3.95 (m, 10H, H-2^{II-V}, H-3^{I-VI}), 3.86–4.74 (m, 13H, H-2^{I}, H-4^{I-VI}, H-5^{I-VI}), 3.59–3.54 (m, 1H, H-2^{VI}, H-$4'^{I-VI}$, H-$1''_a$), 3.46 (m, 1H, H-$1''_b$), 3.40 (s, 3H, OCH_3), 3.34 (t, J = 6.0 Hz, H-6″), 2.95 (t, J = 6.0 Hz, H-7″), 2.20 (t, J = 7.6 Hz, H-5″), 1.98–1.92 (m, 6H, H-$3'_a$ $^{I-VI}$), 1.80–1.72(m, 6H, H-$3'_b{}^{I-VI}$), 1.52 (m, 4H, H-2″ and H-4″), 1.27 (m, 2H, H-3″), 1.12–1.08 (m, 18H, H-6^{I-VI}). ^{13}C NMR (100 MHz, D_2O) δ: 100.8– 98.3 (6C, C-1^{I-VI}), 78.8, 77.7, 77.4, 77.1 (2C), 68.9 (6C, C-$2'^{I-VI}$), 57.8 (6C, C-$4'^{I-VI}$), 16.9–16.7 (6C, C-6^{I-VI}); TOF–MS *m*/*z*: 1670.8 $[M]^+$.

4.9. 5-(Methoxycarbonyl)pentyl 3-*O*-benzyl-4-(2,4-di-*O*-acetyl-3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-2-*O*-levulinoyl-α-D-mannopyranosyl-(1→2)-tetrakis[-3-*O*-benzyl-4-(2,4-di-*O*-acetyl-3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-α-D-mannopyranosyl]-3-*O*-benzyl-4-(2,4-di-*O*-acetyl-3deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-α-D-mannopyranoside (26)

A solution of TMSOTf in toluene (0.03 M, 2 mL) was added with stirring and exclusion of moisture to a mixture of **20** (500 mg, 0.22 mmol), **10** (300 mg, 0.44 mmol), and 4 Å molecular sieves (300 mg) in dichloromethane (10 mL). The stirring was continued overnight, when TLC (2:1 toluene–acetone) showed that only small amount of the imidate remained unchanged. After neutralization with Et_3N (0.2 mL), filtration, and concentration of the filtrate, the residue was chromatographed (3:1 toluene–acetone) to give **26** (450 mg, 73%). ^{1}H NMR (600 MHz, $CDCl_3$) δ: 6.24–5.93 (m, 5H, 5NH), 5.40 (br s, 1H, H-2^{VI}), 5.20–5.15 (m, 6H, H-$2'^{I-VI}$), 5.05–4.97 (3 s, 4H, H-1^{II-V}), 4.69 (s, 2H, H-$1^{I,VI}$), 4.63–4.43 (m, 12H, $6PhCH_2$), 4.30–4.01 (m, 22H, H-2^{II-V}, H-4^{I-VI}, H-$4'^{I-VI}$), 3.86 (br s, 1H, H-2^{I}), 3.80–3.60 (m, 16H, H-3^{I-VI}, H-5^{I-VI}, OCH_3, H-$1''_a$), 3.35 (m, 1H, H-$1''_b$), 2.72–2.61 (m, 4H, CH_2CH_2), 2.33 (t, J = 7.2 Hz, H-$5''_{a,b}$), 2.26–1.99 (m, 51H, $13COCH_3$, H-$3'^{I-VI}$), 1.67–1.55 (m, 4H, H-$2''_{a,b}$, H-$4''_{a,b}$), 1.43–1.31 (m, 2H, H-$3''_{a,b}$), 1.17–1.08 (m, 18H, H-6^{I-VI}); ^{13}C NMR (150 MHz, $CDCl_3$) δ: 137.9 (C_q), 137.8 (C_q), 137.7 (C_q), 137.6 (C_q), 137.5 (C_q), 137.4 (C_q), 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 127.8, 127.6, 127.4, 100.5–99.6 (4C, C-1^{II-V}), 98.6 (2C, C-$1^{I,VI}$), 18.1–17.8 (6C, C-6^{I-VI}); TOF–MS *m*/*z*: 1387 $[M]^{2+}$, 2772 $[M+H]^+$, 2795 $[M+Na]^+$.

4.10. 5-(Methoxycarbonyl)pentyl 4-(3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-α-D-mannopyranosyl-(1→2)-tetrakis[-4-(3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-α-D-mannopyranosyl]-4-(3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-α-D-mannopyranoside (29)

A solution of compound **26** (400 mg, 0.14 mmol) in DCM–MeOH (1:10, 10 mL) was stirred under H_2 with 5% Pd/C (400 mg) overnight, then filtered and concentrated. Without further purification, the residue was treated with 1.0 M NaOMe (1.0 mL) in MeOH (10 mL) to complete deacylation. After neutralization with Amberlite IR-120, H^+-resin, the mixture was filtered, the filtrate was concentrated, and the residue was chromatographed (11 DCM–MeOH) to afford the known[7] compound **29** (210 mg, 89%).

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