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Simultaneous 2-O-deacetylation and 4-amination of peracetylated Neu5Ac: application to the synthesis of (4→4)-piperazine derivatives linked sialic acid dimers

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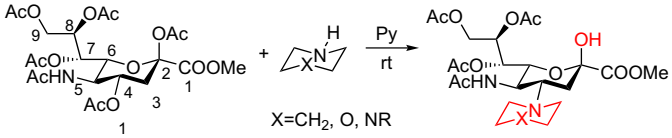
ABSTRACT

A simultaneous stereoselective 2-O-deacetylation and 4-amination reaction of peracetylated Neu5Ac **1** has been established with cyclic secondary amines, such as 1-*N*-Boc-piperazine. Four C_2-symmetric and two asymmetric sialic acid dimers with (4→4)-piperazine derivatives linked were synthesized. They may serve as precursors of unnatural polysialic acids.

1. Introduction

Sialic acids are a family of nine-carbon carboxylated saccharides and play significant roles in a number of important biological processes including cell-to-cell recognition, cell-adhesion, and tumor metastasis.[1] Among the 43 naturally occurring derivatives of sialic acids, *N*-acetylneuraminic acid (Neu5Ac) is a prominent one and present in a variety of glycosidic linkages, most typically α(2→8) or α(2→9) linkages in polysialic acids (PSAs).[2] Naturally occurring PSAs are expressed widely in bacteria where they function as virulence factors.[3] Structural mimicry of PSAs may result in immune tolerance, attenuation host–tumor, and host–pathogen immune reactions.[4] Over the years considerable attention has been paid to the development of methodologies and strategies for efficient α-sialoside installation for the synthesis of these complex sialoconjugates.[5,6] However, the glycosidic oxygen linkage in PSAs is susceptible to the enzymatic action of extracellular sialidases,[7] thus a nor-*C*-linked α(2→8) Neu5Ac disaccharide[8] and several (1→5)-amide linked Neu5Ac dimer derivatives[9] have been synthesized to enhance immunogencity or to be of utility in understanding biological recognition at the molecular level.[10]

We recently discovered an efficient reaction for the simultaneous stereoselective 2-O-deacetylation and 4-amination of peracetylated Neu5Ac **1** with cyclic secondary amines with retention configuration (Scheme 1).[11] The approach involves the formation of oxazolinium intermediate followed by an intermolecular S_N2 reaction to afford the products. It provides high yield and stereoselectivity with only one step, especially when the traditional method[12] requires many tedious protection–deprotection and oxidation–reduction steps.



Scheme 1. 2-O-Deacetylation and 4-amination of peracetylated Neu5Ac **1** with cyclic secondary amines in one pot.

In an effort to explore more structural mimicry of PSAs for their important biological roles, we expand our reaction to stereoselectively synthesize several (4→4)-piperazine derivatives linked sialic acid dimers, which may serve as precursors of unnatural PSAs.

2. Results and discussion

Four C_2-symmetric (**4**, **7**, **8**, and **11**) and two asymmetric (**14** and **15**) sialic acid dimers that employed different piperazine derivatives as linkages were synthesized and the results are summarized in Table 1. As shown in Scheme 2, peracetylated Neu5Ac **1**[13] was initially 2-*O*-deacetylated and 4-aminated with 1-*N*-Boc-piperazine to give the key intermediate **2** in 80% yield. The relative structure of **2** that contained a molecule of water as solvate was confirmed by X-ray crystallographic analysis (Fig. 1).[14] Subsequent deprotectection of Boc group with TFA produced the intermediate **3** in quantitative yield. Finally, coupling of **3** with **1** under the same

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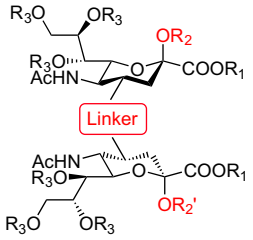
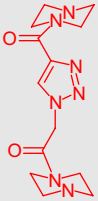
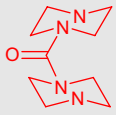
Table 1
Synthesis of sialic acid dimers employing different Linkers



sialic acid dimer

Entry	Linker	R_1	R_2/R_2'	R_3	Dimer: yield[a] (%)
1		Me	$R_2=R_2'=OH$	OAc	**4**: 29
2		Me	$R_2=R_2'=OH$	OAc	**7**: 14,[b] 51[c]
3		H	$R_2=R_2'=OH$	OH	**8**: 46
4		Me	$R_2=R_2'=OAc$	OAc	**11**: 54
5		Me	$R_2=R_2'=OH$	OAc	**14**: 56
6		Me	$R_2=OH$, $R_2'=OAc$	OAc	**15**: 39

[a] Total yield from peracetylated Neu5Ac **1**.
[b] Obtained from 4-amination of compound **1** with amine **6**.
[c] Obtained from compound **3** coupled with triphosgene.

mechanism afforded dimer **4** in 37% yield after 24 h. Prolonging the time for 3 days only increased the yield to 42%. The relatively lower yield may be attributed to the steric hindrance of amine **3**. Dimer **4** was prepared using the same procedure to prepare **2**. It appeared as C_2-symmetry and was free to rotate about the C_2-axis as shown in Scheme 2. Comparison of the ^{1}H NMR spectra of **3** and **4** shows that the chemical shifts and coupling constants, which are characteristic to diagnostic of the sialic acid conformations, are very similar (Fig. 2A and 2B). The ^{1}H and ^{13}C NMR spectra of the two sialic acid parts of **4** overlap with each other.

Treatment of intermediate **3** with 1-*N*-Boc-piperazine in the presence of triphosgene at −10 °C afforded compound **5**. After deprotection of Boc group, **5** was transformed to bulky amine **6**, which subsequently 2-*O*-deacetylated and 4-aminated with compound **1** to furnish dimer **7** in only 28% yield. Alternately, dimer **7** was more efficiently afforded in 65% yield by direct treatment of **3** with triphosgene at 0 °C. Global deprotection of dimer **7** to give dimer **8** proceeded smoothly according to the general procedure.[5a,15] To explore alkyl amines as linkages, C_2-symmetric dimer **11** was obtained using 1,3-dibromopropane as a coupling reagent. Attempts to directly react **3** with 1,3-dibromopropane resulted in a complicated mixture. Under capping the free hydroxyl at C-2 of **3** with an acetyl group, compound **10** was obtained, and then transformed to dimer **11** in 71% yield. Similar to the symmetric dimer **4**, the NMR spectra of the two sialic acid parts of **7** and **11** overlap with each other as well. Notably, the configuration of dimer **11** was determined by empirical rules,[6a,16] since the signals of the equatorially oriented protons at C-3 of Neu5Ac derivatives show a quite characteristic dependence on the anomeric configuration and the chemical shifts of H-3eq and H-3′eq of β-anomers are more upfield (δ=2.30 ppm) than that of α-anomers (δ=2.70 ppm) (Fig. 2C).

In order to study the difference of NMR spectra between symmetric and asymmetric sialic acid dimer derivatives, we firstly synthesized dimer **14** whose asymmetry was attributed to the asymmetric linker through the route outlined in Scheme 3. Compound **1** reacted with 1-*N*-propiolyl-piperazine or 1-*N*-(2-azidoacetyl)-piperazine in Py to furnish building blocks **12** and **13** in 75% and 70% yields, respectively. Dimer **14** was generated by reacting **12** with **13** using the click chemistry method[17] in 80% yield. The two sialic acids' signals of **14** were observed to overlap with each other, same as that of the symmetric dimers, except the methyl esters' hydrogen signals at C-1/C-1′ (3.776 and 3.785 ppm, respectively, S29 in Supplementary data) and the carbon signals at C-4/C-4′ (62.653 and 62.749 ppm, S30 in Supplementary data). This outcome was probably due to the two same sialic acid chains of **14** in a nearly same chemical environment. To confirm this, another asymmetric dimer **15** with different sialic acid moieties was obtained from blocks **3** and **10**. In the ^{1}H NMR spectrum of **15** as illustrated in Figure 2D, the proton signals of the two sialic acid parts were found to be sensitive to the effect of the substituent of sialic acid ring. Thus, the chemical shifts of the H-6, H-8, and H-9 protons of 2-position acetylated sialic acid part were found to be shifted upfield and the H-3eq shifted downfield (Fig. 2D). These data unambiguously confirmed the asymmetry of dimer **15**. The NMR studies indicate two features: (1) the proton signals of the two sialic acid parts of C_2-symmetric dimers overlap with each other (Fig. 2B and 2C) compared with that of asymmetric dimers (Fig. 2D); (2) the proton signals of the two sialic acid parts of **15** show significant difference compared with that of dimer **14**, which is due to the different sialic acid chains and not due to the asymmetric linker part.

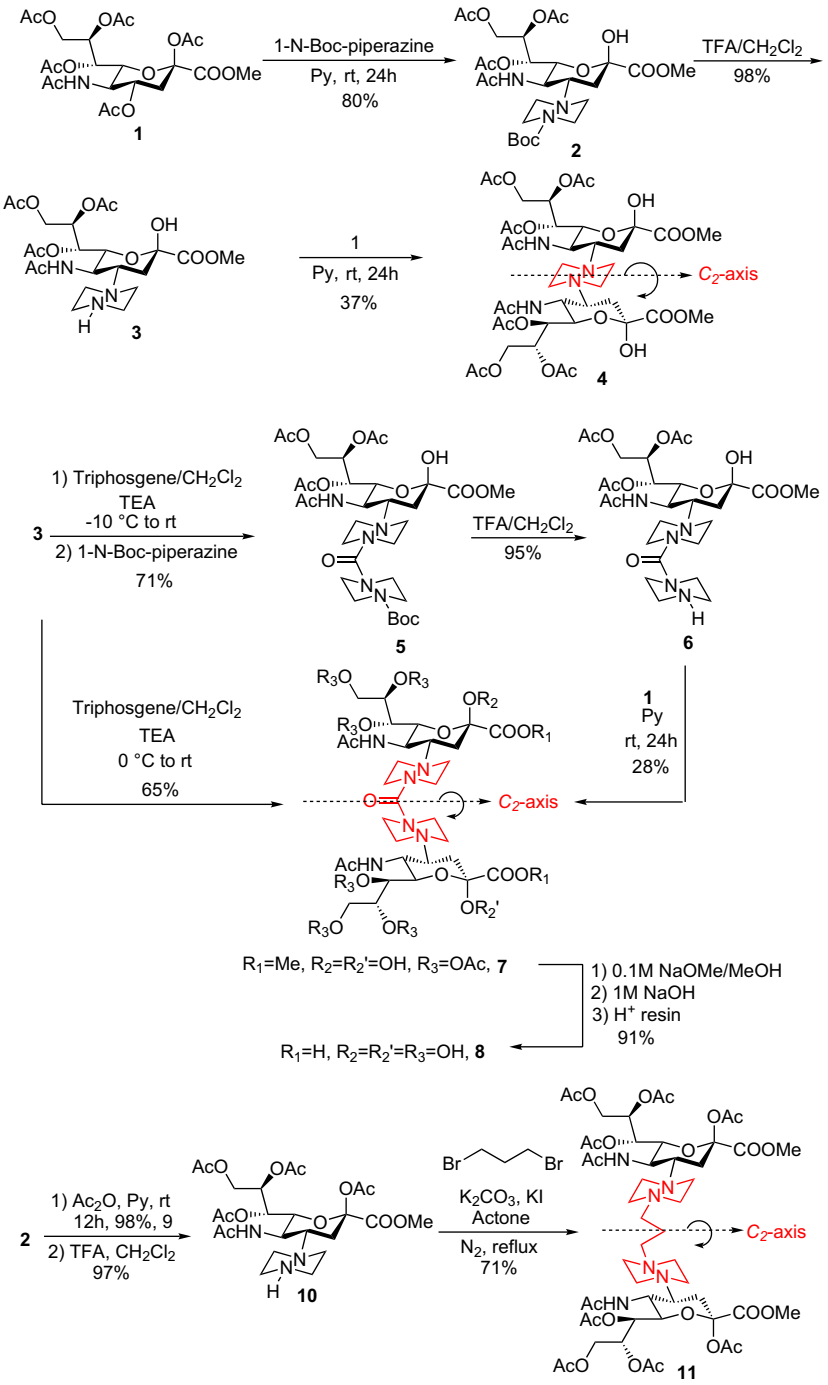
3. Conclusion

In conclusion, we have demonstrated a convenient and highly stereoselective protocol for the synthesis of several novel (4→4)-piperazine derivatives linked sialic acid dimers. Four C_2-symmetric and two asymmetric dimers were synthesized based on the simultaneous stereoselective 2-O-deacetylation and 4-amination of peracetylated Neu5Ac **1** with 1-*N*-Boc-piperazine. With these dimers as precursors, further studies on the free –OH at C-2/C-2′ for selective sialation to synthesize structural mimicry of PSAs are underway in our laboratory.

4. Experimental section

4.1. General methods

All the reagents were used as-obtained, unless otherwise stated. Solvents were evaporated under reduced pressure and below 40 °C if no description was noted. Melting points were measured in a capillary tube without correction. Analytical thin layer chromatography (TLC) was performed on HSGF 254 (0.15–0.2 mm thickness, Yantai Huiyou Company, PR China). ^{1}H and ^{13}C NMR spectra were recorded on a 300 MHz instrument (operated at 300 and 75 MHz, respectively). Chemical shifts were reported in parts per million (ppm, δ) upfield from TMS. Proton coupling patterns were described as singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m), and broad (br). High resolution mass spectra (HRMS) were



Scheme 2. Synthesis of C_2-symmetric dimers **4**, **7**, **8**, and **11**.

obtained on a Fourier Transform Mass Spectrometer. Optical rotations were measured on a polarimeter.

4.2. Methyl 5-acetamido-2-hydroxy-4-(4-*tert*-butoxycarbonyl-piperazin-1-yl)-7,8,9-tri-*O*-acetyl-3,5-dideoxy-β-L-*glycero*-D-*galacto*-2-nonulopyranosidonate (2)

To a stirred solution of peracetylated sialic acid derivatives (**1**) (1.00 g, 1.88 mmol, 1.00 equiv) in dry Py (10 mL) was added 1-*N*-Boc-piperazine (3.50 g, 18.8 mmol, 10.0 equiv) at room temperature (rt). After 24 h, the solvent was evaporated under vacuum below 40 °C, and the residue was dissolved with 30 mL of ethyl acetate, then washed with water (10 mL×3) and brine, and then dried over anhydrous Na_2SO_4. The solvent was removed and the residue was purified by flash chromatography (SiO_2, CH_2Cl_2/MeOH 25:1) to give **2** as a white solid (928 mg, 80%, mp 194–196 °C). $[\alpha]_D^{23}$ +49.2 (*c* 0.50, MeOH). ^{1}H NMR δ (300 MHz, CD_3OD, ppm): 1.43 (s, 9H, $C(CH_3)_3$), 1.78–1.87 (m, 4H, H3a, C5–Ac), 1.96–2.01 (m, 4H, H3e, C9–Ac), 2.02 (s, 3H, C8–Ac), 2.06 (s, 3H, C7–Ac), 2.30–2.35 (br, 2H), 2.65–2.72 (br, 2H), 3.04 (m, 1H, H-4), 3.22–3.31 (br, 4H), 3.78 (s, 3H, OMe), 3.94–4.11 (m, 2H, H-9, H-5), 4.20 (dd, *J*=9.9, 2.1 Hz, 1H, H-6), 4.55 (dd, *J*=12.3, 2.1 Hz, 1H, H-9), 5.13–5.18 (m, 1H, H-8), 5.40–5.42 (m, 1H, H-7); ^{13}C NMR δ (75 MHz, CD_3OD, ppm): 21.1, 21.3, 21.3, 23.2, 29.2 (CH_3 of Boc), 32.3 (C3), 48.2 (C5), 49.5 (CH_2 of piperizine), 53.7 (OMe), 62.7 (C4), 64.3 (C9), 71.0 (C7), 73.5 (C8), 73.6 (C6), 81.6 (C of Boc), 97.0 (C2), 157.0 (CO of Boc), 172.0, 172.4, 172.6, 173.0,

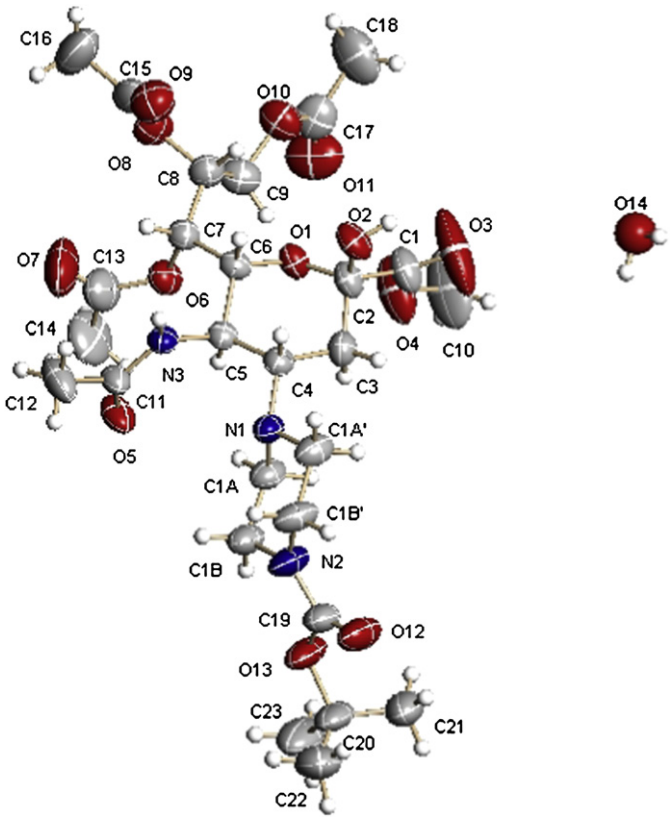


Figure 1. X-ray crystal structure of compound **2**·H_2O.

173.8; ESI-MS *m*/*z* 618 $[M+H]^+$ 100%. HRMS (ESI) calcd for $C_{27}H_{44}N_3O_{13}$ $[M+H]^+$ 618.2874, found 618.2903.

4.3. Methyl 5-acetamido-2-hydroxy-4-(piperazin-1-yl)-7,8,9-tri-*O*-acetyl-3,5-dideoxy-β-L-*glycero*-D-*galacto*-2-nonulopyranosidonate (3)

To a solution of **2** (500 mg, 0.81 mmol) in dry CH_2Cl_2 (10 mL) at room temperature was added TFA (2 mL). The reaction mixture was stirred at room temperature for 2 h. After evaporation of the solvent, 10 mL of MeOH was added. The solution was treated with Dowex Marathon A (OH^-) anion-exchange resin, filtered, and evaporated to give a white foam (410 mg, 98%). $[\alpha]_D^{23}$ +31.2 (*c* 0.47, MeOH). ^{1}H NMR δ (300 MHz, CD_3OD, ppm): 1.81–1.90 (m, 4H, H3a, C5-Ac), 1.96–2.07 (m, 10H, H3e, C9-Ac), 2.54–2.58 (br, 2H), 2.95–3.11 (m, 7H), 3.78 (s, 3H, OMe), 3.91–4.10 (m, 2H, H-9, H-5), 4.20 (dd, *J*=10.2, 2.1 Hz, 1H, H-6), 4.55 (dd, *J*=12.3, 2.1 Hz, 1H, H-9), 5.13–5.18 (m, 1H, H-8), 5.41–5.44 (m, 1H, H-7); ^{13}C NMR δ (75 MHz, CD_3OD, ppm): 21.1, 21.3, 21.4, 23.3, 32.3 (C3), 45.1, 46.3, 47.5 (CH_2 of piperizine), 48.0 (C5), 53.7 (OMe), 62.9 (C4), 64.3 (C9), 70.9 (C7), 73.3 (C8), 73.3 (C6), 96.9 (C2), 171.8, 172.3, 172.6, 173.0, 174.0; ESI-MS *m*/*z* 518 $[M+H]^+$ 100%.

4.4. Methyl 5-acetamido-2-hydroxy-4-(4-(methyl 5-acetamido-2-hydroxy-7,8,9-tri-*O*-acetyl-3,5-dideoxy-β-L-*glycero*-D-*galacto*-2-nonulopyranosidonate-4-yl)-piperazin-1-yl)-7,8,9-tri-*O*-acetyl-3,5-dideoxy-β-L-*glycero*-D-*galacto*-2-nonulopyranosidonate (4)

Compound **4** was obtained using the same procedure to prepare **2** as a white solid after the purification by flash chromatography (SiO_2, CH_2Cl_2/MeOH 15:1) (yield, 37%, mp 137–139 °C). $[\alpha]_D^{23}$ +29.2 (*c* 0.50, MeOH). ^{1}H NMR δ (300 MHz, CD_3OD, ppm): 1.82–1.90 (m, 8H, H3a, H3a′, C5–Ac, C5′–Ac), 1.99–2.08 (m, 20H, H3e, H3e′, OAc×6), 2.44–2.51 (m, 4H), 2.84–2.68 (m, 4H), 3.05 (m, 2H, H-4, H-4′), 3.78 (s, 6H, OMe×2), 3.94–4.09 (m, 4H, H-9, H-9′, H-5, H-5′), 4.20 (dd, *J*=9.9, 2.1 Hz, 2H, H-6, H-6′), 4.55 (dd, *J*=12.0, 2.4 Hz, 2H, H-9), 5.13–5.17 (m, 2H, H-8, H-8′), 5.38–5.41 (m, 2H, H-7, H-7′); ^{13}C NMR δ (75 MHz, CD_3OD, ppm): 21.1, 21.3, 21.3, 23.5, 32.5 (C3, C3′), 48.0 (C5, C5′), 49.5 (CH_2 of piperizine), 53.7 (OMe, OMe′), 62.9 (C4, C4′), 64.3 (C9, C9′), 70.8 (C7, C7′), 73.4 (C8, C8′), 73.4 (C6, C6′), 96.8 (C2, C2′), 171.7, 172.4, 172.6, 173.0, 174.0; ESI-MS *m*/*z* 949 $[M+H]^+$ 100%. HRMS (ESI) calcd for $C_{40}H_{61}N_4O_{22}$ $[M+H]^+$ 949.3777, found 949.3769.

4.5. Methyl 5-acetamido-2-hydroxy-4-(4-(4-*tert*-butoxycarbonyl-piperazin-1-carbonyl)-piperazin-1-yl)-7,8,9-tri-*O*-acetyl-3,5-dideoxy-β-L-*glycero*-D-*galacto*-2-nonulopyranosidonate (5)

To a stirred solution of triphosgene (40 mg, 0.13 mmol) and Et_3N (128 μL, 0.92 mmol) in dry CH_2Cl_2 (5 mL) at −10 °C was added **3** (200 mg, 0.39 mmol) dropwise in dry CH_2Cl_2 (2 mL). After 30 min, 1-*N*-Boc-piperazine (72 mg, 0.39 mmol) was added, and the reaction mixture was allowed to warm to room temperature, stirred for additional 2 h, diluted with CH_2Cl_2, washed with water and brine, and dried over Na_2SO_4. The solvent was removed and the residue was purified by flash chromatography (SiO_2, CH_2Cl_2/MeOH 22:1) to give **5** as a white solid (202 mg, 71%, mp 205–206 °C). $[\alpha]_D^{23}$ +42.9 (*c* 0.42, MeOH/CH_2Cl_2=1:1). ^{1}H NMR δ (300 MHz, $CDCl_3$, ppm): 1.46 (s, 9H, $C(CH_3)_3$), 1.91–1.97 (m, 4H, H-3a, Ac), 2.03–2.14 (m, 10H, H-3e, Ac×3), 2.34–2.37 (br, 2H), 2.72–2.76 (br, 2H), 2.99 (br, 1H, H-4), 3.18–3.25 (br, 8H), 3.38–3.41 (br, 4H), 3.80 (s, 3H, OMe), 3.97–4.04 (m, 1H, H-9), 4.06–4.16 (m, 2H, H-6, H-5), 4.49 (dd, *J*=12.0, 2.4 Hz, 1H, H-9), 5.18–5.24 (m, 1H, H-8), 5.34–5.38 (m, 1H, H-7); ^{13}C NMR δ (75 MHz, CD_3OD, ppm): 21.1, 21.4, 21.4, 23.3, 29.1 (CH_3 of Boc), 32.2 (C3), 48.2 (C5), 48.4, 48.9, 49.9 (CH_2 of piperizine), 53.7 (OMe), 62.7 (C4), 64.3 (C9), 71.0 (C7), 73.5 (C8), 73.6 (C6), 82.0 (C of Boc), 97.0 (C2), 156.9 (CO of Boc), 166.0 (CO of ureas), 172.0, 172.4, 172.6, 173.0, 173.8; ESI-MS *m*/*z* 752 $[M+Na]^+$ 100%. HRMS (ESI) calcd for $C_{32}H_{51}N_5O_{14}Na$ $[M+Na]^+$ 752.3330, found 752.3316.

4.6. Methyl 5-acetamido-2-hydroxy-4-(4-(piperazin-1-carbonyl)piperazin-1-yl)-7,8,9-tri-*O*-acetyl-3,5-dideoxy-β-L-*glycero*-D-*galacto*-2-nonulopyranosidonate (6)

Compound **6** was obtained using the same procedure to prepare **3** as a white solid (yield, 95%, mp 124–126 °C). $[\alpha]_D^{23}$ +40.0 (*c* 0.47, MeOH). ^{1}H NMR δ (300 MHz, $CDCl_3$, ppm): 1.93–1.97 (m, 4H, H-3a, Ac), 2.04–2.13 (m, 10H, H3e, Ac×3), 2.31–2.37 (br, 2H), 2.68–2.76 (br, 2H), 2.82–2.89 (m, 4H), 2.98 (br, 1H, H-4), 3.14–3.26 (br, 8H), 3.38–3.41 (br, 4H), 3.87 (s, 3H, OMe), 3.97–4.16 (m, 3H, H-9, H-6, H-5), 4.49 (dd, *J*=12.6, 2.4 Hz, 1H, H-9), 5.17–5.24 (m, 1H, H-8), 5.34–5.38 (dd, *J*=5.7, 1.5 Hz, 1H, H-7); ^{13}C NMR δ (75 MHz, CD_3OD, ppm): 21.1, 21.4, 21.4, 23.2, 32.2 (C3), 46.4, 48.2 (C5), 48.7, 48.9, 49.9 (CH_2 of piperizine), 53.7 (OMe), 62.7 (C4), 64.3 (C9), 71.0 (C7), 73.5 (C8), 73.6 (C6), 97.0 (C2), 166.0 (CO of ureas), 172.0, 172.4, 172.6, 173.0, 173.8; ESI-MS *m*/*z* 630 $[M+H]^+$ 100%. HRMS (ESI) calcd for $C_{27}H_{44}N_5O_{12}$ $[M+H]^+$ 630.2986, found 630.3003.

4.7. Methyl 5-acetamido-2-hydroxy-4-(4-(4-(methyl 5-acetamido-2-hydroxy-7,8,9-tri-*O*-acetyl-3,5-dideoxy-β-L-*glycero*-D-*galacto*-2-nonulopyranosidonate-4-yl)piperazin-1-carbonyl)piperazin-1-yl)-7,8,9-tri-*O*-acetyl-3,5-dideoxy-β-L-*glycero*-D-*galacto*-2-nonulopyranosidonate (7)

To a stirred solution of compound **3** (100 mg, 0.19 mmol) in CH_2Cl_2 (5 mL) at 0 °C were added Et_3N (40 μL, 0.29 mmol) and triphosgene (10 mg, 0.033 mmol) in dry CH_2Cl_2 (0.50 mL). The

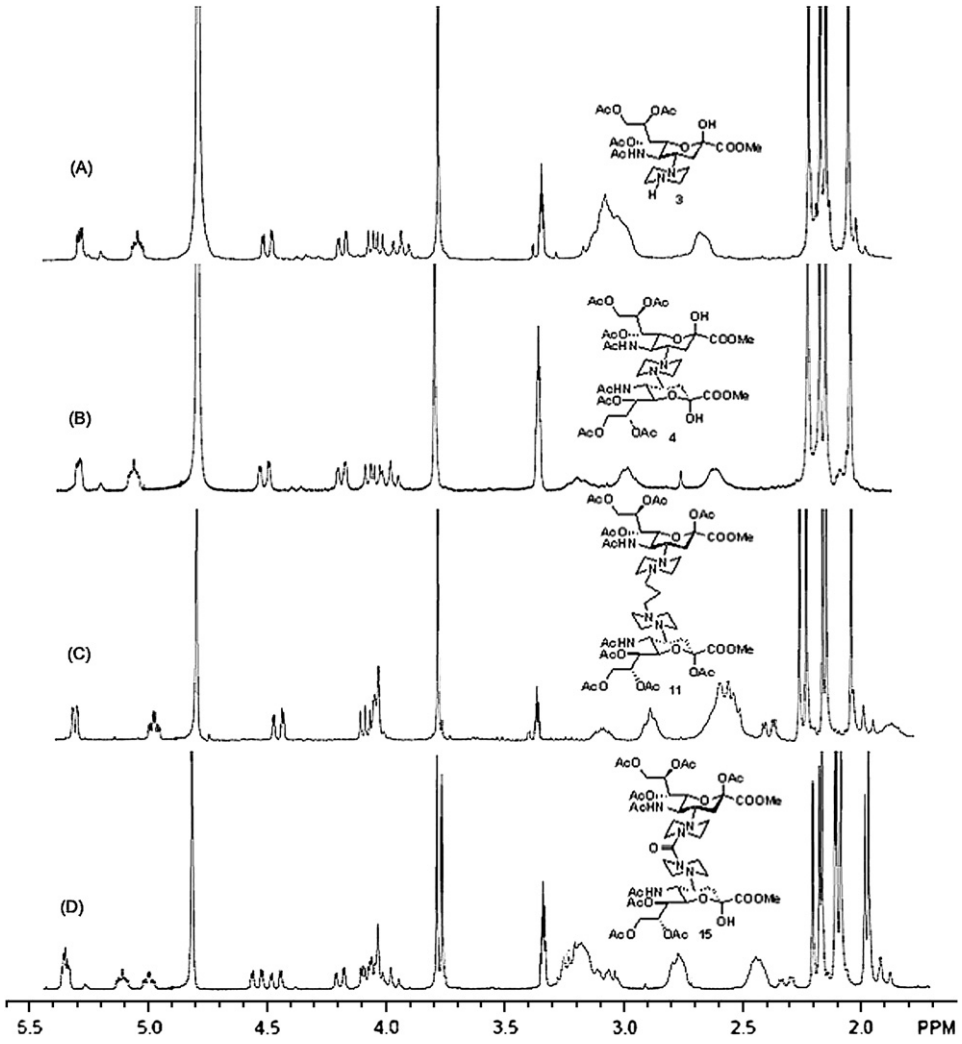


Figure 2. Comparison of the ^{1}H NMR spectrum of compound **3** (A), C_2-symmetric dimers **4** (B) and **11** (C), and asymmetric dimer **15** (D).

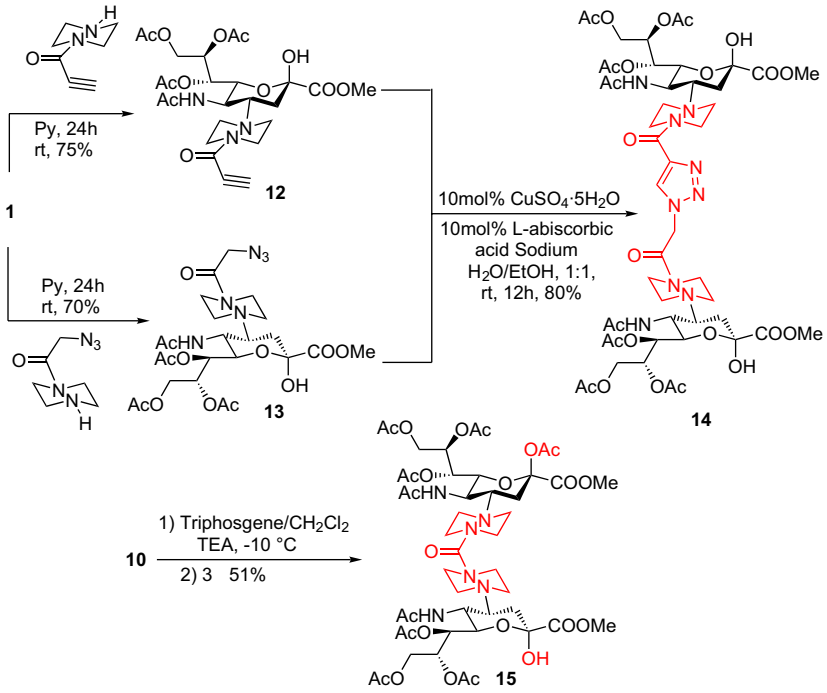
reaction mixture was then allowed to warm to room temperature, stirred for an additional 2 h, diluted with CH_2Cl_2, washed with H_2O and brine, and dried over Na_2SO_4. The solvent was removed and **7** was obtained as a white solid after the purification by flash chromatography (SiO_2, CH_2Cl_2/MeOH 12:1) (yield, 65%, mp 145–146 °C). $[\alpha]_D^{23}$ +57.8 (*c* 0.51, MeOH). ^{1}H NMR δ (300 MHz, CD_3OD, ppm): 1.79–1.87 (m, 8H), 2.00–2.06 (m, 20H), 2.35–2.38 (br, 4H), 2.71–2.74 (br, 4H), 3.03–3.24 (m, 10H), 3.77 (s, 6H), 3.94–4.10 (m, 4H), 4.20 (dd, *J*=10.5, 2.4 Hz, 2H), 4.55 (dd, *J*=12.3, 2.4 Hz, 2H), 5.13–5.18 (m, 2H), 5.40–5.42 (dd, *J*=5.7, 2.4 Hz, 2H); ^{13}C NMR δ (75 MHz, CD_3OD, ppm): 21.2, 21.4, 21.4, 23.2, 32.1 (C3, C3′), 48.1 (C5, C5′), 48.4, 49.2, 49.9 (CH_2 of piperizine), 53.7 (OMe, OMe′), 62.7 (C4, C4′), 64.3 (C9, C9′), 71.0 (C7, C7′), 73.5 (C8, C8′), 73.6 (C6, C6′), 97.0 (C2, C2′), 166.0 (CO of ureas), 171.9, 172.4, 172.6, 173.0, 173.8; ESI-MS *m*/*z* 1083 $[M+Na]^+$ 100%. HRMS (ESI) calcd for $C_{45}H_{68}N_6O_{23}Na$ $[M+Na]^+$ 1083.4234, found 1083.4203.

4.8. 5-Acetamido-2-hydroxy-4-(4-(4-(5-acetamido-2-hydroxy-7,8,9-tri-hydroxy-3,5-dideoxy-β-L-*glycero*-D-*galacto*-2-nonulopyranosidonic-4-yl)piperazin-1-carbonyl)-piperazin-1-yl)-7,8,9-tri-hydroxy-3,5-dideoxy-β-L-*glycero*-D-*galacto*-2-nonulopyranosidonic acid (8)

To a solution of **7** (50 mg) in MeOH (5 mL), 0.1 M NaOMe/MeOH was added until the reaction mixture was about pH 7. After stirring for 2 h, 1 M NaOH was added and the resulting mixture was stirred overnight. Dowex 50 (H^+) cation-exchange resin was added, and the resulting mixture was stirred, filtered, and then evaporated to dryness (yield, 91%). ^{1}H NMR δ (300 MHz, CD_3OD, ppm): 1.93 (t, *J*=12.6 Hz, 2H), 2.05–2.15 (m, 8H), 2.60–2.70 (br, 4H), 2.81–2.93 (br, 4H), 3.26 (m, 2H), 3.52–3.56 (dd, *J*=9.3, 0.9 Hz, 2H), 3.60–3.66 (m, 2H), 3.71–3.81 (m, 12H), 4.04–4.07 (dd, *J*=10.2, 1.2 Hz, 2H), 4.20–4.27 (t, *J*=10.2 Hz, 2H); ESI-MS *m*/*z* 803 $[M+Na]^+$ 100%. HRMS (ESI) calcd for $C_{31}H_{52}N_6O_{17}Na$ $[M+Na]^+$ 803.3287, found 803.3265.

4.9. Methyl 5-acetamido-2-acetoxy-4-(4-*tert*-butoxycarbonyl-piperazin-1-yl)-7,8,9-tri-*O*-acetyl-3,5-dideoxy-β-L-*glycero*-D-*galacto*-2-nonulopyranosidonate (9)

To a stirred solution of compound **2** (200 mg, 0.32 mmol) and DMAP (2 mg for catalysis) in dry Py (5 mL), Ac_2O was added (33 μL, 0.35 mmol) dropwise at room temperature. After stirring overnight, the solvent was evaporated under vacuum below 40 °C and the residue was purified by flash chromatography (SiO_2, CH_2Cl_2/MeOH 30:1) to give **9** as a white foam (204 mg, 97%). $[\alpha]_D^{23}$ +16.7 (*c* 0.49, MeOH). ^{1}H NMR δ (300 MHz, CD_3OD, ppm): 1.36 (s, 9H, $C(CH_3)_3$), 1.76 (t, *J*=12.9 Hz, 1H, H-3a), 1.86 (s, 3H), 1.97 (s, 3H), 1.99 (s, 3H), 2.05 (s, 3H), 2.06 (s, 3H), 2.18–2.26 (m, 3H), 2.61–2.65 (br, 2H), 2.94–3.03 (m, 1H, H-4), 3.17–3.30 (br, 4H), 3.72 (s, 3H, OMe), 3.95–4.11 (m, 3H, H-9, H-5, H-6), 4.45 (dd, *J*=12.3, 2.1 Hz, 1H, H-9), 4.94–4.96 (m, 1H, H-8), 5.33–5.35 (m, 1H, H-7); ^{13}C NMR δ (75 MHz, CD_3OD, ppm): 21.2, 21.2, 21.4, 21.4, 23.3, 29.1 (CH_3 of Boc), 32.0 (C3), 47.6 (C5), 49.5



Scheme 3. Synthesis of asymmetric dimers **14** and **15**.

(CH_2 of piperizine), 53.9 (OMe), 62.5 (C4), 63.7 (C9), 70.1 (C7), 72.8 (C8), 75.3 (C6), 81.6 (C of Boc), 99.6 (C2), 156.9 (CO of Boc), 169.4, 170.6, 172.1, 172.1, 172.9, 173.8; ESI-MS *m*/*z* 660 $[M+H]^+$ 100%.

4.10. Methyl 5-acetamido-2-acetoxy-4-(piperazin-1-yl)-7,8,9-tri-*O*-acetyl-3,5-dideoxy-β-L-*glycero*-D-*galacto*-2-nonulopyranosidonate (10)

Compound **10** was obtained using the same procedure to prepare **3** as a white foam (yield, 97%). $[\alpha]_D^{23}$ +10.8 (*c* 0.50, MeOH). ^1H NMR δ (300 MHz, CD_3OD, ppm): 1.84 (t, *J*=12.9 Hz, 1H, H-3a), 1.90 (s, 3H), 2.00 (s, 3H), 2.02 (s, 3H), 2.07 (s, 3H), 2.12 (s, 3H), 2.26 (dd, *J*=13.5, 3.9 Hz, 1H, H-3e), 2.40–2.45 (br, 2H), 2.77–2.88 (br, 6H), 2.97 (m. 1H, H-4), 3.77 (s, 3H, OMe), 4.03–4.10 (m, 3H, H-9, H-5, H-6), 4.46 (dd, *J*=12.6, 2.4 Hz, 1H, H-9), 5.01–5.05 (m, 1H, H-8), 5.30–5.41 (m, 1H, H-7); ^{13}C NMR δ (75 MHz, CD_3OD, ppm): 21.2, 21.2, 21.3, 21.4, 23.3, 31.7 (C3), 47.2, 47.4 (C5), 49.7 (CH_2 of piperizine), 53.9 (OMe), 62.7 (C4), 63.7 (C9), 70.1 (C7), 72.7 (C8), 75.3 (C6), 99.7 (C2), 169.4, 170.6, 172.1, 172.1, 172.9, 173.8; ESI-MS *m*/*z* 560 $[M+H]^+$ 100%. HRMS (ESI) calcd for $C_{24}H_{38}N_3O_{12}$ $[M+H]^+$ 560.2455, found 560.2403.

4.11. Methyl 5-acetamido-2-acetoxy-4-(4-(4-(methyl 5-acetamido-2-acetoxy-7,8,9-tri-*O*-acetyl-3,5-dideoxy-β-L-*glycero*-D-*galacto*-2-nonulopyranosidonate-4-yl)piperazin-1-propyl)piperazin-1-yl)-7,8,9-tri-*O*-acetyl-3,5-dideoxy-β-L-*glycero*-D-*galacto*-2-nonulopyranosidonate (11)

To a stirred solution of compound **7** (150 mg, 0.27 mmol), K_2CO_3 (41 mg, 0.30 mmol), and KI (5 mg for catalysis) under an atmosphere of nitrogen in dry acetone (5 mL), 1,3-dibromopropane (15 μL, 0.15 mmol) was added at room temperature. The reaction mixture was then warmed to reflux and kept at reflux overnight. After it was cooled to room temperature, the undissolved salts were removed by filtration through Celite and the filtrate was concentrated. The residue was purified by flash chromatography (SiO_2, CH_2Cl_2/MeOH 12:1) to give **11** as a light yellow foam (111 mg, 71%). $[\alpha]_D^{23}$ +17.8 (*c* 0.50, MeOH). ^1H NMR δ (300 MHz, CD_3OD, ppm): 1.70–1.73 (m, 2H), 1.83 (t, *J*=13.2 Hz, 2H, H-3a, H-3a′), 1.90 (s, 6H), 2.00 (s, 6H), 2.01 (s, 6H), 2.09 (s, 6H), 2.12 (s, 6H), 2.30 (dd, *J*=13.5, 3.9 Hz, 2H, H-3e, H-3e′), 2.39–2.48 (br, 16H), 2.7–2.82 (br, 4H), 3.00 (m. 2H, H-4), 3.75 (s, 6H, OMe), 4.02–4.10 (m, 6H, H-9, H-9′, H-5, H-5′, H-6, H-6′), 4.46 (dd, *J*=13.2, 2.1 Hz, 2H, H-9, H-9′), 5.01–5.05 (m, 2H, H-8, H-8′), 5.30–5.40 (m, 2H, H-7, H-7′); ^{13}C NMR δ (75 MHz, CD_3OD, ppm): 21.2, 21.2, 21.3, 21.4, 23.3, 31.8 (C3, C3′), 47.6 (C5, C5′), 53.9 (OMe, OMe′), 55.3, 57.8 (CH_2 of piperizine), 62.1 (C4, C4′), 63.7 (C9, C9′), 70.1 (C7, C7′), 72.7 (C8, C8′), 75.4 (C6, C6′), 99.7 (C2, C2′), 169.4, 170.6, 172.1, 172.1, 172.9, 173.8; ESI-MS *m*/*z* 1159 $[M+H]^+$ 100%. HRMS (ESI) calcd for $C_{51}H_{78}N_6O_{24}Na$ $[M+Na]^+$ 1181.4965, found 1181.4994.

4.12. Methyl 5-acetamido-2-hydroxy-4-(4-propioloyl-piperazin-1-yl)-7,8,9-tri-*O*-acetyl-3,5-dideoxy-β-L-*glycero*-D-*galacto*-2-nonulopyranosidonate (12)

Compound **12** was obtained using the same procedure to prepare **2** as a white solid after the purification by flash chromatography (CH_2Cl_2/MeOH 20:1) (yield, 75%, mp 121–123 °C). $[\alpha]_D^{23}$ +47.3 (*c* 0.51, MeOH). ^1H NMR δ (300 MHz, CD_3OD, ppm): 1.84–1.92 (m, 4H), 2.00–2.06 (m, 10H), 2.45–2.55 (br, 2H), 2.80–2.94 (br, 2H), 3.11–3.20 (m, 1H), 3.55 (br, 2H), 3.64–3.82 (m, 5H), 3.97–4.10 (m, 2H), 4.23 (dd, *J*=10.2, 2.4 Hz, 1H), 4.55 (dd, *J*=12.3, 2.4 Hz, 1H), 5.13–5.18 (m, 1H), 5.41–5.44 (m, 1H); ^{13}C NMR δ (75 MHz, CD_3OD, ppm): 21.1, 21.3, 21.4, 23.3, 32.3 (C3), 43.5, 48.1 (C5), 48.9, 50.1, 50.3 (CH_2 of piperizine), 53.7 (OMe), 63.0 (C4), 64.3 (C9), 70.8 (C7), 73.3 (C8), 73.3 (C6), 76.3 (CH of propioloyl), 83.2, 96.9 (C2), 154.2 (CO of propioloyl), 171.8, 172.3, 172.5, 173.0, 174.1; ESI-MS *m*/*z* 570 $[M+H]^+$ 100%. HRMS (ESI) calcd for $C_{25}H_{36}N_3O_{12}$ $[M+H]^+$ 570.2299, found 570.2315.

4.13. Methyl 5-acetamido-2-hydroxy-4-(4-(2-azidoacetyl)-piperazin-1-yl)-7,8,9-tri-*O*-acetyl-3,5-dideoxy-β-L-*glycero*-D-*galacto*-2-nonulopyranosidonate (13)

Compound **13** was obtained using the same procedure to prepare **2** as a white solid after purification by flash chromatography

(CH_2Cl_2/MeOH 20:1) (yield, 70%, mp 155–156 °C). $[\alpha]_D^{23}$ +50.7 (*c* 0.58, MeOH). ^{1}H NMR δ (300 MHz, CD_3OD, ppm): 1.76–1.87 (t, *J*=12.6 Hz, 1H), 1.89 (s, 3H), 1.97–2.0 (m, 10H), 2.35–2.45 (br, 2H), 2.70–2.80 (br, 2H), 3.00–3.08 (m, 1H), 3.24–3.37 (br, 2H), 3.45–3.53 (m, 2H), 3.77 (3, 3H), 3.94–4.10 (m, 4H), 4.21 (dd, *J*=10.2, 2.4 Hz, 1H), 4.56 (dd, *J*=12.3, 2.4 Hz, 1H), 5.13–5.18 (m, 1H), 5.40–5.43 (m, 1H); ^{13}C NMR δ (75 MHz, CD_3OD, ppm): 21.2, 21.4, 21.4, 23.2, 32.2 (C3), 44.4, 47.3, 48.1 (C5), 50.0, 50.1 (CH_2 of piperizine), 52.1 (CH_2 of 2-azidoacetyl), 53.7 (OMe), 62.7 (C4), 64.3 (C9), 70.6 (C7), 73.4 (C8), 73.5 (C6), 97.0 (C2), 168.7 (CO of 2-azidoacetyl), 171.9, 172.3, 172.5, 173.0, 173.8; ESI-MS *m*/*z* 601 $[M+H]^+$ 100%. HRMS (ESI) calcd for $C_{24}H_{37}N_6O_{12}$ $[M+H]^+$ 601.2469, found 601.2457.

4.14. Methyl 5-acetamido-2-hydroxy -4-(4-(((4-(methyl 5-acetamido-2-hydroxy-7,8,9-tri-*O*-acetyl-3,5-dideoxy-β-L-*glycero*-D-*galacto*-2-nonulopyranosidonate-4-yl)-piperazin-1-yl)-2-oxoethyl)-1*H*-1,2,3-triazole-4-carbonyl)piperazin-1-yl)-7,8,9-tri-*O*-acetyl-3,5-dideoxy-β-L-*glycero*-D-*galacto*-2-nonulopyranosidonate (14)

Compounds **12** (100 mg, 0.18 mmol) and **13** (105 mg, 0.18 mmol) were suspended in a 1:1 mixture of water and ethanol (8 mL). Sodium ascorbate (0.018 mmol, 18 μL of freshly prepared 1 M solution in water) was added followed by $CuSO_4 \cdot 5H_2O$ (4.5 mg, 0.018 mmol, in 20 μL of water). The heterogeneous mixture was stirred vigorously overnight. The solvent was removed under vacuum below 40 °C and **14** was obtained as a light yellow solid after the purification by flash chromatography (SiO_2, CH_2Cl_2/MeOH 12:1) (168 mg, 80%, mp 130–132 °C). $[\alpha]_D^{23}$ +48.0 (*c* 0.24, MeOH). ^{1}H NMR δ (300 MHz, CD_3OD, ppm): 1.85–1.94 (m, 8H, H-3a, H-3a′, Ac×2), 2.00–2.09 (m, 20H, H-3e, H-3e′, Ac×6), 2.41–2.47 (br, 4H), 2.75–2.84 (br, 4H), 3.07–3.10 (m, 2H, H-4, H-4′), 3.44–3.72 (br, 8H), 3.78 (d, *J*=2.7 Hz, 6H, OMe, OMe′), 4.00–4.10 (m, 4H), 4.21–4.24 (d, *J*=10.5 Hz, 2H), 4.55–4.59 (d, *J*=12.0 Hz, 2H), 5.17–5.18 (m, 2H), 5.42–5.44 (m, 2H), 5.50 (s, 2H), 8.27 (s, 1H); ^{13}C NMR δ (75 MHz, CD_3OD, ppm): 21.1, 21.4, 21.4, 23.3, 32.2 (C3, C3′), 44.7, 47.4, 48.2 (C5, C5′), 49.7, 50.1 (CH_2 of piperizine), 52.5 (CH_2 of 2-azidoacetyl), 53.7 (OMe, OMe′), 62.7 (C4, C4′), 64.3 (C9, C9′), 71.0 (C7, C7′), 73.5 (C8, C8′), 73.6 (C6, C6′), 97.0 (C2, C2′), 131.3 (CH of triazole), 144.5 (C of triazole), 162.7, 166.3, 172.0, 172.5, 172.6, 173.1, 173.9; ESI-MS *m*/*z* 1192 $[M+Na]^+$ 100%. HRMS (ESI) calcd for $C_{49}H_{71}N_9O_{24}Na$ $[M+Na]^+$ 1192.4510, found 1192.4559.

4.15. Methyl 5-acetamido-2-acetoxy-4-(4-(4-(methyl 5-acetamido-2-hydroxy-7,8,9-tri-*O*-acetyl-3,5-dideoxy-β-L-*glycero*-D-*galacto*-2-nonulopyranosidonate-4-yl)-piperazin-1-propyl)piperazin-1-yl)-7,8,9-tri-*O*-acetyl-3,5-dideoxy-β-L-*glycero*-D-*galacto*-2-nonulopyranosidonate (15)

To a stirred solution of triphosgene (19 mg, 0.06 mmol) and Et_3N (57 μL, 0.42 mmol) in dry CH_2Cl_2 (5 mL) at −10 °C was added **10** (100 mg, 0.18 mmol) dropwise in dry CH_2Cl_2 (2 mL). After 30 min, compound **3** (93 mg, 0.18 mmol) was added, and the reaction mixture was allowed to warm to room temperature, stirred for additional 2 h, diluted with CH_2Cl_2, washed with water and brine, and dried over Na_2SO_4. The solvent was removed and the residue was purified by flash chromatography to give **15** as a white solid (SiO_2, CH_2Cl_2/MeOH 15:1) (yield, 51%, mp 147–149 °C). $[\alpha]_D^{23}$ +41.6 (*c* 0.50, MeOH). ^{1}H NMR δ (300 MHz, CD_3OD, ppm): 1.77–1.87 (m, 5H, H-3a, H-3a′, Ac), 1.89 (s, 3H, Ac), 1.97–2.05 (m, 13H, H-3e, Ac×4), 2.08 (s, 3H, Ac), 2.09 (s, 3H, Ac), 2.12 (s, 3H, Ac), 2.25 (dd, *J*=13.5, 3.9 Hz, 1H, H-3e′), 2.35–2.38 (br, 4H), 2.65–2.74 (br, 4H), 2.98–3.00 (m, 2H, H-4, H-4′), 3.14–3.20 (br, 8H), 3.75 (s, 3H), 3.77 (s, 3H), 3.94–4.11 (m, 5H), 4.20 (dd, *J*=10.2, 2.1 Hz, 1H), 4.49 (dd, *J*=12.3, 2.4 Hz, 1H), 4.55 (dd, *J*=10.2, 2.1 Hz, 1H), 2.01–5.05 (m, 1H), 5.13–5.18 (m, 1H), 5.37–5.42 (m 2H). ^{13}C NMR δ (75 MHz, CD_3OD, ppm): 21.2, 21.4, 23.2 and 23.3, 31.9 and 32.1 (C3, C3′), 47.5, 48.1 (C5, C5′), 48.4, 49.1, 49.9 (CH_2 of piperizine), 53.7, 53.9 (OMe, OMe′), 62.5, 62.7 (C4, C4′), 63.7, 64.3 (C9, C9′), 70.1, 71.0 (C7, C7′), 72.8, 73.5 (C8, C8′), 75.3 (C6, C6′), 97.0, 99.6 (C2, C2′), 166.0 (CO of ureas), 169.4, 170.6, 172.0,172.1, 172.4, 172.6, 172.9, 173.0, 173.8; ESI-MS *m*/*z* 1125 $[M+Na]^+$ 100%. HRMS (ESI) calcd for $C_{47}H_{70}N_6O_{24}Na$ $[M+Na]^+$ 1125.4339, found 1125.4314.

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Supplementary data

Copies of ^{1}H and ^{13}C NMR spectra for all compounds and X-ray data of compound **2** can be found in the online version. Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2008.04.046.

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14. CCDC 658252 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.
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