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Transsialidase from *Trypanosoma cruzi* for Regio- and Stereoselective Synthesis of N-Acyl-Modified Sialylated Oligosaccharides and Measurement of Transfer Rates

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Abstract: Recombinant transsialidase from *Trypanosoma cruzi* (TcTS) was used for the sialylation with natural and non-natural derivatives of neuraminic acid. Neu5Ac-α(2→3)-Gal-β(1→6)-Glc-αOMe was prepared in 80% yield. Correspondingly, the modified trisaccharide derivatives Neu5Prop-α(2→3)-Gal-β(1→6)-Glc-αOMe (32%) and Neu5Gc-α(2→3)-Gal-β(1→6)-Glc-αOMe (Prop = propanoyl, Gc = glycolyl) were obtained in 60% yield, respectively.

Keywords: enzyme catalysis · glycosylation · neuraminic acid · sialylation · transsialidase

Introduction

Neuraminic acid (Neu5Ac) plays an important role in nature as a major constituent of a variety of glycoconjugates (such as oligosaccharides, glycoproteins and gangliosides) occurring in animals and several pathogens.[1] In most cases, neuraminic acids are terminally α(2→3) or α(2→6)-linked to a galactose of the oligosaccharide cell epitope. Due to the terminal position of Neu5Ac in these oligosaccharide scaffolds, the sialylation is associated with many processes, such as cell-recognition and cell differentiation. Neu5Ac allows recognition by a suitable receptor protein; on the other hand, the presence of Neu5Ac is able to mask recognition sites.

The application of the reversible nature of glycosidases in the synthesis of various oligosaccharides presents a facile glycosylation procedure. Sialidases from several sources were exploited in transsialylations as their acceptance of artificial donor glycosides, such as *p*NP-αNeu5Ac (*p*NP = *p*-nitrophenyl) and MU-αNeu5Ac, has been recognized.

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In Chagas disease, transsialidase from *Trypanosoma cruzi* (TcTS) causes the transfer of Neu5Ac from a human host cell to the cell epitope of the pathogen. This unusual transfer mechanism enables the pathogen to protect its own cell surface against recognition by the human immune system. Interestingly, this enzyme belongs to the superfamily of sialidases but shows merely transferase activity in the presence of a suitable acceptor molecule. Thus, transsialidase allows for transglycosylation of natural and non-natural donor substrates, such as *p*NP-Neu5Ac to Galβ-R acceptor structures, leading to a large variety of complex and biologically active oligosaccharides. Previously, a series of terminally sialylated oligosaccharides could be obtained, and subsequently used as building blocks for convenient syntheses of more complex glycoconjugates in good yields.[2]

Neu5Gc, which is a foreign neuraminic acid derivative to humans because of an inactivating mutation of the gene encoding the enzyme CMP-*N*-acetylneuraminic acid (CMP-Neu5Ac) hydroxylase was found to be synthesized by many cancerous tissues as a tumor-associated Hanganutziu–Deicher antigen.[3] The mutation responsible for the absence of Neu5Gc in humans occurred after our last common ancestor with bonobos and chimpanzees, and before the origin of present-day humans, prior to brain expansion.[4] Thus, it could be of medical and even paleontological interest to have the possibility to synthesize Neu5Gc-containing model structures. In this case, the use TcTS offers a good solution, utilising its stereo- and regioselectivity.

N-Acyl-modified sialic acids, such as Neu5Prop were previously reported to be obtained by an enzymatic approach by Kayser et al. and other groups.[5–10] Herein, we report the chemical synthesis of N-acyl-modified neuraminic acid

donors by using the method of Komba et al.[11] and a highly regio- and stereoselective enzymatic transglycosylation with TcTS to Gal-β(1→6)-Glc-αOMe.

Results and Discussion

Previously the transfer of side-chain-shortened neuraminic acid derivatives to a variety of different acceptor molecules was reported with good yields.[2,12] Figure 1 shows TcTS in a complex with the excellent donor-structure sialyllactose.[13] Lactose is fixed by Tyr_{119} and Trp_{312} and the carboxyl group of Neu5Ac by Arg_{314}. The C7–C9 side chain of the substrate is located outside the binding pocket, and this may be the reason for the high tolerance of the enzyme towards changes of this moiety. In contrast, the acetyl group is located deep in the pocket, fixed by Asp_{96}. Thus, an acyl modification should have stronger effects on the transfer process of the protein, and enzymatic synthesis of sialylated oligosaccharides with acyl modifications by using TcTS should be challenging. By use of molecular modelling with SYBYL we arrived at the assumption that there should be space for elongation of the acetyl substituent by at least one CH_3 group.

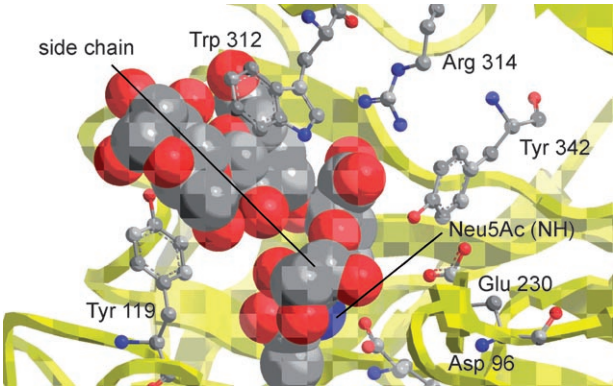
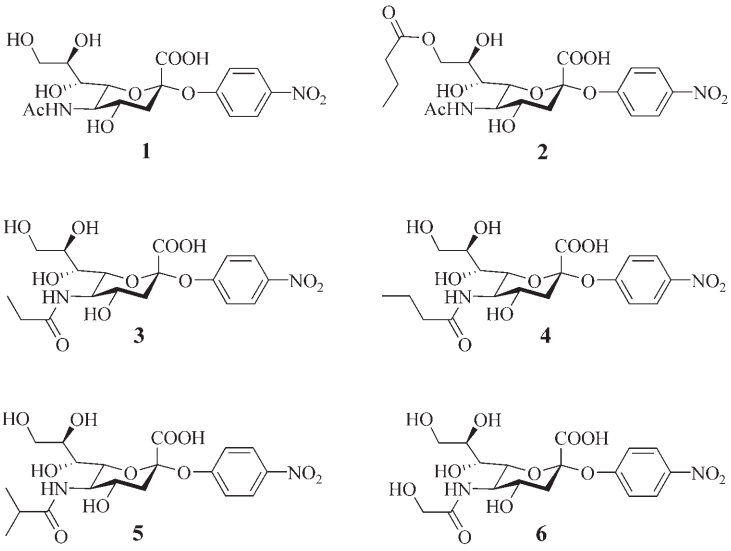


Figure 1. Neu5Ac lactose in complex with TcTS; PDB-Code = 1S0.

Synthesis of donor substrates: According to the donor specificity, *p*NP-Neu5Ac derivatives could be used as non-natural sialyl-donor structures, leading to α(2→3)-linked sialylated oligosaccharides. In contrast to natural donor substrates, the use of non-natural ones has the advantage that a strict kinetic control to fix the equilibrium of sialyllactose and product is not needed as the free phenolic compound *p*NP-OH is not a suitable acceptor.[14] Six potential donor derivatives were synthesised chemically. The most challenging problem in the synthesis of compounds **3** to **6** is the selective protection of the amino group. Further, care must be taken

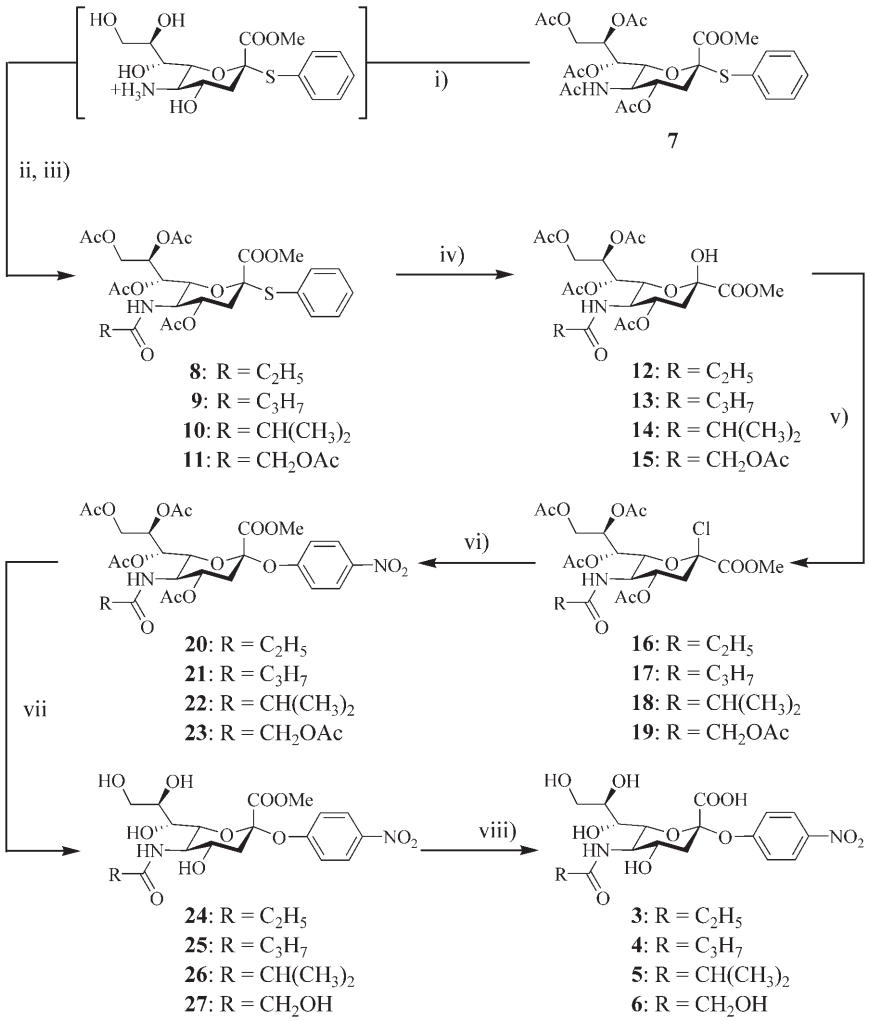


to prevent neuraminic acid from formation of a five-membered ring between the amino and the keto group during deprotection. Ring formation was avoided by protecting neuraminic acid from ring opening by formation of the thiophenyl glycoside which proved to be stable even under strongly acidic conditions. Following the method of Rothermel et al., **7** was prepared in good yield.[15] Deprotection with methanesulfonic acid and selective reprotection were carried out in methanol following the method of Sugata et al.[16] The use of alcohol as the solvent has the advantage that no strict control of temperature and concentration are needed, as the 5-amino function of neuraminic acid is nucleophilic enough to withstand the solvent competition. In contrast, the hydroxyl groups were not acylated in the presence of methanol. By using pyridine as the solvent, higher acylated byproducts were obtained.

After acidic deprotection of **7**, Et_3N and two equivalents of the corresponding anhydride were added and stirred for 1 h at 0 °C. In contrast to the use of *N*-hydroxysuccinimide esters,[16] the use of an anhydride was easier and therefore preferred in the synthesis of glycoyl derivative **11**.

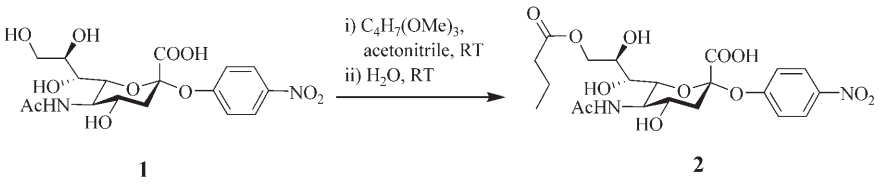
The corresponding anhydride was easy to synthesise by starting with the commercially available acetoxyacetyl chloride, following the method of Plusquellec et al.[17] The solution was evaporated and the residue was acetylated in pyridine with acetic anhydride to give components **8**–**11**. Hydrolysis of these thioglycosides by using NBS in acetone/water at room temperature gave the hemiacetales **12**–**15** (Scheme 1).

Despite the fact that protons at C-3 loose their characteristic positions in ^1H NMR spectra, the stereochemistry at the anomeric position was easy to determine because of the



Scheme 1. i) MsOH, MeOH, reflux; ii) Et_3N, $(RCO)_2O$, 0 °C; iii) Ac_2O, pyridine; iv) acetone, NBS, H_2O; v) AcCl, MeOH; vi) *p*NP-OH, 1 N NaOH, dichloromethane, 3 h; vii) MeOH, NaOMe; viii) 0.1 N NaOH. NBS = *N*-bromosuccinimide; Ms = methanesulfonyl.

coupling between H-3_{ax} and the proton of the anomeric hydroxyl group. The subsequent formation of chlorides **16–19**, preparation of corresponding *p*NP glycosides **20–23** by phase-transfer catalysis, Zemplén deacetylation to compounds **24–27** and hydrolysis to give **3–6** could be performed partly in analogy to previous methods.[15] The synthesis of the 9-*O*-acylated derivative **2** started with **1** by using a modified method of Ogura et al.[18] The reaction was carried out in acetonitrile with trimethyl orthobutyrate in the presence of catalytic amounts of *p*-toluenesulfonic acid followed by in situ hydrolysis of the *ortho* ester (Scheme 2). Purification by column chromatography with RP-18 silica gel (water/acetonitrile) under neutral conditions yielded compound **2** in 41 % yield.

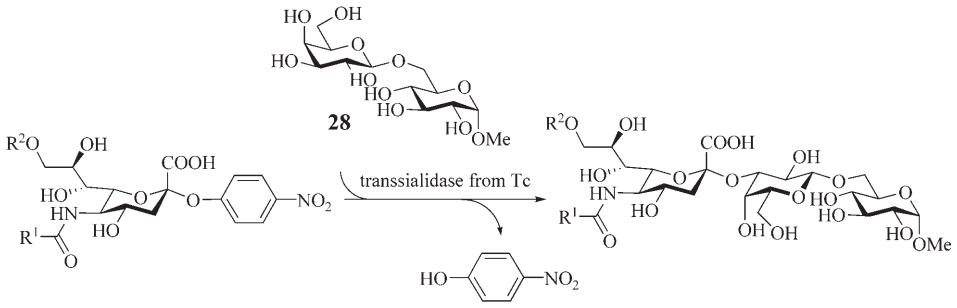


Scheme 2. Selective 9-O-acylation with trimethyl orthobutyrate.

Transsialylation with transsialidase TcTS: The activity of TcTS is difficult to measure and an assay would usually require radioactively labelled material. To avoid this, the quantity was determined by the concentration of the pure enzyme by using the same concentration for each test. This allowed for a comparison of the yields of different transsialylations, following a procedure established by R. Field et al.[19] Potential donor substrates **1–6** were tested for transsialylation with methyl α-allolactoside (**28**), which proved to be an excellent acceptor substrate and was therefore used to avoid limiting effects due to an acceptor substrate. In addition, this was, therefore, taken to observe even marginal transglycosylation activities with non-natural donor substrates, in the presence of TcTS for 17 h, giving yields from 32 to 87 % (Scheme 3). Purification by Biogel (P2) chromatography allowed an easy and fast isolation of products.

Compound **2** could be used for transsialylation comparable to compound **1**. As expected, elongation of the N-acetyl group by one methylene group led to a drastic decrease in yield from 87 to 32 %. Due to the relatively deep location of the acyl group in the transition state (Figure 1), further modifications were challenging; however, potential donor substrates **4** and **5** were not transferred. Despite the steric demand of an additional hydroxyl group, the N-glycolyl derivative **6** turned out to be a suitable donor and allowed the enzymatic transfer with 60 % yield.

The stereochemistry of the linkage was assigned by a characteristic shift of H-3_{eq} and H-3_{ax} of Neu5Acyl due to the anisotropy of the carbonyl group.[20] The regiochemistry of the linkage could be determined by the chemical shift of C-3. After sialylation, this carbon showed a clear downfield shift of approximately $\delta =$ 3.0 ppm compared to the non-



1: $R^1 = CH_3$	$R^2 = H$	**29**: $R^1 = CH_3$	$R^2 = H$	(80 %)
2: $R^1 = CH_3$	$R^2 = C_4H_7O$	**30**: $R^1 = CH_3$	$R^2 = C_4H_7O$	(78 %)
3: $R^1 = C_2H_5$	$R^2 = H$	**31**: $R^1 = C_2H_5$	$R^2 = H$	(32 %)
4: $R^1 = C_3H_7$	$R^2 = H$	**32**: $R^1 = C_3H_7$	$R^2 = H$	(0 %)
5: $R^1 = CH(CH_3)_2$	$R^2 = H$	**33**: $R^1 = CH(CH_3)_2$	$R^2 = H$	(0 %)
6: $R^1 = CH_2OH$	$R^2 = H$	**34**: $R^1 = CH_2OH$	$R^2 = H$	(60 %)

Scheme 3. Transglycosylation of synthesised donor derivatives.

sialylated disaccharide.[2] The transfer rates of the novel donor substrates were measured by recording ^{1}H NMR spectra and subsequent integration of the aromatic proton signals of *p*NP-Neu5Ac derivatives and of the released *p*NP-OH. The incubation of donor and acceptor followed the procedure mentioned above by using D_2O instead of H_2O. To determine the conversion at a given time, 100 μL of incubated solution were transferred into ready NMR tubes containing a mixture of 1:1 D_2O/[D_4]methanol to stop the reaction by denaturing the enzyme. Conversion rates were calculated from the signals of the *p*NP moiety in the starting material *p*NP-Neu5Ac and in the product *p*NP-OH (Figure 2).

By using the described procedure, maximum rates of conversion (V_{max}) and the Michaelis constants (K_M) were obtained by measuring reaction velocities in D_2O at different substrate concentrations. The resulting Lineweaver–Burk plot is shown in Figure 3. Under the described conditions, *p*NP-Neu5Ac showed a K_M of 10 μM and a V_{max} of 91 nmol min^{-1}. By using *p*NP-Neu5Gc as a substrate, K_M increased to 31 μM and V_{max} decreased to 41 nmol min^{-1}. K_M and V_{max} for *p*NP-Neu5Prop were found to be 45 μM and 12 nmol min^{-1}, respectively, and for *p*NP-Neu5Ac9But (But = butanoyl) 10 μM and 51 nmol min^{-1}, respectively.

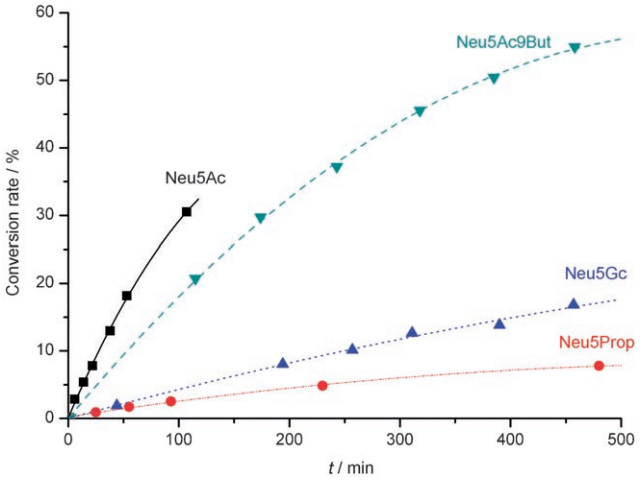


Figure 2. Transsialylation of Neu5Acyl derivatives in D_2O measured by ^{1}H NMR spectroscopy.

Conclusion

Various neuraminic acid derivatives were transferred enzymatically by employing transsialidase from *Trypanosoma cruzi*. Changes of the substituent at nitrogen showed drastic effects related to the transfer ratio. Nevertheless TcTS turned out to be a useful tool for the synthesis of oligosaccharides containing α2→3-linked glycolyl neuraminic acid. Neu5Gc-α(2→3)-Gal-β(1→6)-Glc-αOMe was isolated in 60 % yield. Propanoyl neuraminic acid was still obtained in a yield of 30 %. On the other hand, TcTS showed a high tolerance towards chemical changes of the glycerol side chain.

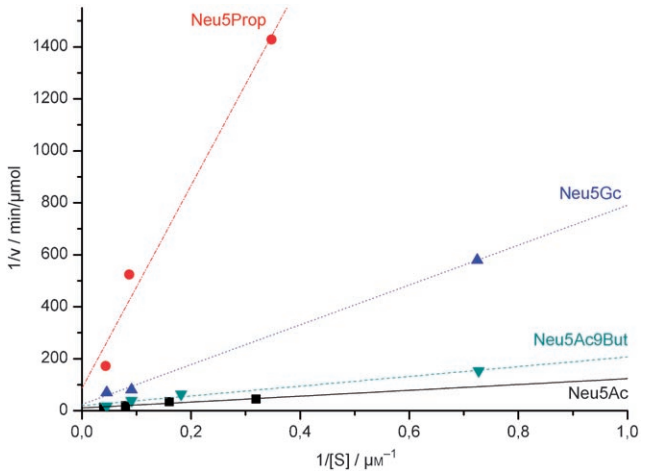


Figure 3. Lineweaver–Burk plot of experimental data measured by ^{1}H NMR spectroscopy.

Experimental Section

General remarks: Commercially available starting materials were used without further purification. Solvents were dried according to standard methods. Purification of the products was carried out by column chromatography by using Merck silica gel 60 (230–400 mesh). The enzymatic reactions were incubated in a thermomixer Comfort (Merck) at 600 rpm. The NMR spectra were recorded on a Bruker AMX-400 (100.62 MHz for ^{13}C NMR spectra, 400.14 MHz for ^{1}H NMR spectra) or DRX-500

(125.77 MHz for ^{13}C NMR spectra, 500.13 MHz for ^{1}H NMR spectra) spectrometer. All chemical shifts are quoted in ppm downfield from TMS or referred to the characteristic signals of the used solvents $CHCl_3$ in $CDCl_3$ (7.26 ppm), $[D_5]C_6H_6$ in $[D_6]C_6H_6$ (7.16 ppm), $[D_3]$MeOH in [D4]MeOH (3.31 ppm) or HDO in D_2O (4.79 ppm). Mass spectra were recorded on a Bruker MALDI-Tof Biflex III.

Transsialylation with transsialidase from *Trypanosoma cruzi* (method 1): A solution of donor (25 μmol) and acceptor (35 μmol) in degassed incubation buffer (1.0 mL, 100 mM Tris/HCl, pH 7.5, 50 mg BSA, 0.02 % NaN_3) was incubated with recombinant TcTS (80 μL, 1.3 mg/1 mL) at 23 °C for 24 h. The reaction was monitored by TLC (butanol/acetic acid/water 5:2:2). After completion, the enzyme was denatured and centrifuged before the supernatant was lyophilized. The dry residue was dissolved in water and purified by biogel chromatography (P-2 Biogel, 16 × 900 mm) with water.

Deacetylation and selective N-acylation and O-acetylation of 7 (method 2): Compound **7** was dissolved in dry methanol (1 mL/100 mg) and methanesulfonic acid (0.1 mL per 100 mg **7**) was added. The solution was heated under reflux for 24 h and was then neutralized with triethylamine (Et_3N). Further triethylamine (0.1 mL per 100 mg of **7**) was added and the solution was treated with the corresponding anhydride (1.5–2.0 equiv) at 0 °C and stirred for 1 h. After this time, the solution was evaporated and the residue dissolved in acetic anhydride. Dry pyridine was added dropwise at 0 °C and stirred for 24 h. The solution was concentrated and the residue was taken-up in chloroform, washed with cold hydrochloric acid (1 M) and water, dried (Na_2SO_4) and then concentrated. The products were purified by column chromatography (ethyl acetate) on silica gel.

Procedure for hydrolysis of neuraminic acid thioglycosides (method 3): NBS (3 equiv) was added to a solution of the thioglycoside in acetone (4 mL per 100 mg thioglycoside) and water (0.2 mL per 100 mg thioglycoside) and the resulting mixture was stirred for 0.5 h at room temperature. After this time, the solution was concentrated, diluted with $CHCl_3$, washed with saturated aqueous $NaHCO_3$, dried ($MgSO_4$) and evaporated. The products were purified by column chromatography (ethyl acetate) on silica gel.

4-Nitrophenyl (5-acetamido-9-*O*-butanoyl-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosylonic acid) (2): *p*NP-Neu5Ac (**1**, 180 mg, 418 μmol) was suspended in acetonitrile (4 mL). Trimethylorthobutyrate (260 μL, 1.67 mmol) and toluene-4-sulfonic acid (2 mg) were added and stirred for 1 h. Subsequently, the intermediate was hydrolysed by the addition of H_2O (1 mL). The solution was evaporated and the residue purified by column chromatography (H_2O/acetonitrile) on RP-18 silica gel. Compound **2** was obtained as a colourless solid. Yield: 86 mg (41 %); m.p. 143 °C; $[\alpha]_{546}^{20} = +217$ ($c = 1$ in H_2O); ^{1}H NMR (400 MHz, D_2O): $\delta = 8.17$ (d, $J_{(Ha,Hb)arom} = 9.2$ Hz, 2H; H-a_{arom}), 7.23 (d, $J_{(Ha,Hb)arom} = 9.2$ Hz, 2H; H-b_{arom}), 4.33 (dd, $J_{8,9a} = 2.3$, $J_{9a,9b} = 11.7$ Hz, 1H; H-9a), 4.19 (dd, $J_{5,6} = 10.4$, $J_{6,7} = 1.3$ Hz, 1H; H-6), 4.17 (dd, $J_{8,9b} = 5.6$, $J_{9a,9b} = 11.7$ Hz, 1H; H-9b), 4.00 (ddd, $J_{7,8} = 9.2$, $J_{8,9a} = 2.3$, $J_{8,9b} = 5.6$ Hz, 1H; H-8), 3.93 (dd, $J_{4,5} = 10.3$, $J_{5,6} = 10.4$ Hz, 1H; H-5), 3.78 (ddd, $J_{3ax,4} = 12.0$, $J_{3eq,4} = 4.6$, $J_{4,5} = 10.3$ Hz, 1H; H-4), 3.59 (dd, $J_{6,7} = 1.3$, $J_{7,8} = 9.2$ Hz, 1H; H-7), 2.79 (dd, $J_{3ax,3eq} = 12.7$, $J_{3eq,4} = 4.6$ Hz, 1H; H-3_{eq}), 2.36 (dd, $J_{CH2,CH2} = 7.1$ Hz, 2H; CH_2(2)-But), 2.02 (s, 3H; $COCH_3$), 1.99 (dd, $J_{3ax,3eq} = 12.7$, $J_{3ax,4} = 12.0$ Hz, 1H; H-3_{ax}), 1.58 (sext, $J_{CH2,CH2} = 7.1$, $J_{CH2,CH3} = 7.4$ Hz, 2H; CH_2(3)-But), 0.88 ppm (dd, $J_{CH2,CH3} = 7.4$ Hz, 3H; CH_3(4)-but); ^{13}C NMR (101 MHz, D_2O): $\delta = 177.27$ (C-1), 160.36 (C-1-but), 125.89 (C-a_{arom}), 120.16 (C-b_{arom}), 102.58 (C-2), 74.04 (C-6), 69.38 (C-8), 68.76 (C-7), 67.94 (C-4), 66.07 (C-9), 52.09 (C-5), 41.26 (C-3), 36.12 (C-2-But), 22.40 ($COCH_3$), 18.41 (C-3-But), 13.18 ppm (C-4-But); MALDI-TOF: *m*/*z* (%): calcd for $C_{21}H_{28}N_2O_{12}$: 500.45; found: 523.0 $[M+Na]^+$.

4-Nitrophenyl (5-*N*-propanoyl-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosylonic acid) (3): Compound **24** (112 mg, 244 μmol) was dissolved in aqueous NaOH (25 mL, 0.1 M). After stirring for 0.5 h, the solution was neutralized with Amberlite IR 120 (H^+) resin and lyophilized. The dry residue was dissolved in water and purified by biogel chromatography (P-2 Biogel, 16 × 900 mm) with water. Compound **3** was obtained as a colourless solid. Yield: 70.5 mg (70 %); m.p. 98 °C; $[\alpha]_{546}^{20} = +70$ ($c = 1$ in H_2O); ^{1}H NMR (400 MHz, D_2O): $\delta = 8.21$ (d, $J_{(Ha,Hb)arom} = 9.4$, 2H; H-a_{arom}), 7.28 (d, $J_{(Ha,Hb)arom} = 9.4$, 2H; H-b_{arom}), 4.16 (dd, $J_{5,6} = 10.4$, $J_{6,7} = 1.5$ Hz, 1H; H-6), 3.96 (dd, $J_{4,5} = 10.5$, $J_{5,6} = 10.4$ Hz, 1H; H-5), 3.86–3.81 (m, 3H; H-4/H-8/H-9a), 3.62 (dd, $J_{8,9b} = 6.6$, $J_{9a,9b} = 12.5$ Hz, 1H; H-9b), 3.56 (dd, $J_{6,7} = 1.5$, $J_{7,8} = 8.9$ Hz, 1H; H-7), 2.84 (dd, $J_{3ax,3eq} = 13.0$, $J_{3eq,4} = 4.8$ Hz, 1H; H-3_{eq}), 2.31 (ddd, $J_{CH2,CH3} = 7.6$ Hz, 2H; CH_2-Prop), 2.02 (dd, $J_{3ax,3eq} = 13.0$, $J_{3ax,4} = 11.7$ Hz, 1H; H-3_{ax}), 1.12 ppm (dd, $J_{(Ha,Hb)arom} = 9.4$ Hz, 3H; CH_3); ^{13}C NMR (101 MHz, D_2O): $\delta = 125.88$ (C-a_{arom}), 120.46 (C-b_{arom}), 102.77 (C-2), 74.12 (C-6), 71.79 (C-4), 68.66 (C-7), 67.97 (C-8), 63.12 (C-9), 51.92 (C-5), 41.31 (C-3), 29.62 (CH_2-Prop), 9.88 ppm (CH_3-Prop); MALDI-TOF: *m*/*z*: calcd for $C_{18}H_{24}N_2O_{11}$: 444.39; found: 466.7 $[M+Na]^+$.

4-Nitrophenyl (5-*N*-butanoyl-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosylonic acid) (4): Compound **25** (99.0 mg, 210 μmol) was dissolved in aqueous NaOH (25 mL, 0.1 M). After stirring for 0.5 h, the solution was neutralized with Amberlite IR 120 (H^+) resin and lyophilized. The dry residue was dissolved in water and purified by biogel chromatography (P-2 Biogel, 16 × 900 mm) with water. Compound **4** was obtained as a colourless solid. Yield: 75.0 mg (78 %); m.p. 85 °C; $[\alpha]_{546}^{20} = +89$ ($c = 1$ in H_2O); ^{1}H NMR (400 MHz, D_2O): $\delta = 8.23$ (d, $J_{(Ha,Hb)arom} = 9.4$ Hz, 2H; H-a_{arom}), 7.30 (d, $J_{(Ha,Hb)arom} = 9.4$ Hz, 2H; H-b_{arom}), 4.17 (dd, $J_{5,6} = 10.2$, $J_{6,7} = 1.3$ Hz, 1H; H-6), 3.98 (dd, $J_{4,5} = 10.2$, $J_{5,6} = 10.2$ Hz, 1H; H-5), 3.88–3.79 (m, 3H; H-4/H-8/H-9a), 3.63 (dd, $J_{8,9b} = 7.0$, $J_{9a,9b} = 12.7$ Hz, 1H; H-9b), 3.59 (dd, $J_{6,7} = 1.3$, $J_{7,8} = 8.9$ Hz, 1H; H-7), 2.82 (dd, $J_{3ax,3eq} = 13.0$, $J_{3eq,4} = 4.6$ Hz, 1H; H-3_{eq}), 2.29 (dd, $J_{CH2,CH2} = 7.4$ Hz, 2H; CH_2-a-But), 2.03 (dd, $J_{3ax,3eq} = 13.0$, $J_{3ax,4} = 11.7$ Hz, 1H; H-3_{ax}), 1.63 (m, 2H; CH_2-b-But), 0.93 ppm (dd, $J_{CH2,CH3} = 7.4$ Hz, 3H; CH_3); ^{13}C NMR (101 MHz, D_2O): $\delta = 125.93$ (C-a_{arom}), 120.30 (C-b_{arom}), 74.13 (C-6), 71.70 (C-4), 68.75 (C-7), 67.94 (C-8), 63.14 (C-9), 52.00 (C-5), 41.39 (C-3), 38.23 (C-2-But), 19.39 (C-3-But), 13.21 ppm (CH_3-But); MALDI-TOF: *m*/*z*: calcd for $C_{19}H_{26}N_2O_{11}$: 458.42; found: 480.8 $[M+Na]^+$.

4-Nitrophenyl (5-*N*-isobutanoyl-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosylonic acid) (5): Compound **26** (50.0 mg, 106 μmol) was dissolved in aqueous NaOH (10 mL, 0.1 M). After stirring for 0.5 h, the solution was neutralized with Amberlite IR 120 (H^+) resin and lyophilized. The dry residue was dissolved in water and purified by biogel chromatography (P-2 Biogel, 16 × 900 mm) with water. Compound **5** was obtained as a colourless solid. Yield: 48.2 mg (100 %); m.p. 118 °C; $[\alpha]_{546}^{20} = +11$ ($c = 1$ in H_2O); ^{1}H NMR (400 MHz, D_2O): $\delta = 8.21$ (d, $J_{(Ha,Hb)arom} = 9.4$, 2H; H-a_{arom}), 7.28 (d, $J_{(Ha,Hb)arom} = 9.4$ Hz, 2H; H-b_{arom}), 4.17 (dd, $J_{5,6} = 10.4$, $J_{6,7} = 1.5$ Hz, 1H; H-6), 3.94 (dd, $J_{4,5} = 10.5$, $J_{5,6} = 10.4$ Hz, 1H; H-5), 3.86–3.80 (m, 3H; H-4/H-8/H-9a), 3.62 (dd, $J_{8,9b} = 6.6$, $J_{9a,9b} = 12.5$ Hz, 1H; H-9b), 3.53 (dd, $J_{6,7} = 1.5$, $J_{7,8} = 8.9$ Hz, 1H; H-7), 2.84 (dd, $J_{3ax,3eq} = 12.8$, $J_{3eq,4} = 4.6$ Hz, 1H; H-3_{eq}), 2.55 (h, $J_{(CH,CH3a)But} = 7.1$, $J_{(CH,CH3b)But} = 7.1$ Hz, 1H; CH-But), 2.02 (dd, $J_{3ax,3eq} = 12.8$, $J_{3ax,4} = 11.7$ Hz, 1H; H-3_{ax}), 1.12 (d, $J_{(CH,CH3a)But} = 7.1$ Hz, 3H; CH_3a-But), 1.11 ppm (d, $J_{(CH,CH3b)But} = 7.1$ Hz, 3H; CH_3b-But); ^{13}C NMR (101 MHz, D_2O): $\delta = 125.91$ (C-a_{arom}), 120.26 (C-b_{arom}), 102.68 (C-2), 74.14 (C-6), 71.73 (C-4), 68.70 (C-7), 67.78 (C-8), 63.10 (C-9), 51.81 (C-5), 41.35 (C-3), 35.58 (CH-But), 19.32 (CH_3a-But), 18.79 ppm (CH_3b-But); MALDI-TOF: *m*/*z*: calcd for $C_{19}H_{26}N_2O_{11}$: 458.42; found: 481.1 $[M+Na]^+$.

4-Nitrophenyl (5-*N*-glycolyl-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosylonic acid) (6): Compound **27** (18 mg, 39 μmol) was dissolved in aqueous NaOH (5 mL, 0.1 M). After stirring for 0.5 h, the solution was neutralized with Amberlite IR 120 (H^+) resin and lyophilized. The dry residue was dissolved in water and purified by biogel chromatography (P-2 Biogel, 16 × 900 mm) with water. Compound **6** was obtained as a colourless solid. Yield: 48.2 mg (98 %); m.p. 123 °C; $[\alpha]_{546}^{20} = +83$ ($c = 1$ in H_2O); ^{1}H NMR (400 MHz, D_2O): $\delta = 8.24$ (d, $J_{(Ha,Hb)arom} = 9.3$ Hz, 2H; H-a_{arom}), 7.31 (d, $J_{(Ha,Hb)arom} = 9.3$ Hz, 2H; H-b_{arom}), 4.28 (dd, $J_{5,6} = 10.4$, $J_{6,7} = 1.3$ Hz, 1H; H-6), 4.15 (s, 2H; CH_2-Gc), 4.06 (dd, $J_{4,5} = 10.2$, $J_{5,6} = 10.4$ Hz, 1H; H-5), 3.93 (ddd, $J_{3ax,4} = 12.2$, $J_{3eq,4} = 4.6$, $J_{4,5} = 10.2$ Hz, 1H; H-4), 3.89–3.84 (m, 2H; H-8/H-9a), 3.65 (dd, $J_{8,9b} = 6.6$, $J_{9a,9b} = 12.5$ Hz, 1H; H-9b), 3.60 (dd, $J_{6,7} = 1.3$, $J_{7,8} = 8.9$ Hz, 1H; H-7), 2.88 (dd, $J_{3ax,3eq} = 12.7$, $J_{3eq,4} = 4.6$ Hz, 1H; H-3_{eq}), 2.05 ppm (dd, $J_{3ax,3eq} = 12.7$, $J_{3ax,4} = 12.2$ Hz, 1H; H-3_{ax}); ^{13}C NMR (101 MHz, D_2O): $\delta = 125.93$ (C-a_{arom}), 120.32 (C-b_{arom}), 102.77 (C-2), 73.85 (C-6), 71.82 (C-4), 68.54 (C-7), 67.83 (C-8), 63.12 (C-9), 61.36 (CH_2-Gc), 51.77 (C-5), 41.27 ppm (C-3);

MALDI-TOF: *m/z*: calcd for $C_{17}H_{22}N_2O_{12}$: 446.36; found: 469.3 $[M+Na]^+$.

Thiophenyl (methyl-5-*N*-propanoyl-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosylonate) (8): Compound **7** (500 mg, 857 μmol) was converted into the *N*-propanoyl derivative **8** by following method 2 with propionic anhydride (200 μL, 1.55 mmol). Compound **8** was obtained as an amorphous solid. Yield: 176 mg (34%); $[\alpha]_{546}^{20}=+13$ ($c=0.5$ in $CHCl_3$); ^{1}H NMR (400 MHz, C_6D_6): $\delta=7.63$ (dd, $J_{H\text{-}m,H\text{-}o}=8.4$, $J_{H\text{-}o,H\text{-}p}=1.3$ Hz, 2H; H-o), 7.16 (dd, $J_{H\text{-}m,H\text{-}o}=8.4$, $J_{H\text{-}m,H\text{-}p}=7.3$ Hz, 2H; H-m), 7.08 (m, 1H; H-p), 5.64 (ddd, $J_{7,8}=6.5$, $J_{8,9a}=2.3$, $J_{8,9b}=5.8$ Hz, 1H; H-8), 5.54 (dd, $J_{6,7}=2.0$, $J_{7,8}=6.5$ Hz, 1H; H-7), 4.90 (ddd, $J_{3ax,4}=11.8$, $J_{3eq,4}=4.9$, $J_{4,5}=10.3$ Hz, 1H; H-4), 4.75 (dd, $J_{8,9a}=2.3$, $J_{9a,9b}=12.3$ Hz, 1H; H-9a), 4.59 (d, $J_{5,NH}=10.3$ Hz, 1H; NH), 4.47 (dd, $J_{8,9b}=5.8$, $J_{9a,9b}=12.3$ Hz, 1H; H-9b), 4.38 (ddd, $J_{4,5}=10.3$, $J_{5,6}=10.3$, $J_{5,NH}=10.3$ Hz, 1H; H-5), 4.01 (dd, $J_{5,6}=10.3$, $J_{6,7}=2.0$ Hz, 1H; H-6), 3.24 (s, 3H; $COOCH_3$), 2.97 (dd, $J_{3ax,3eq}=12.8$, $J_{3eq,4}=4.9$ Hz, 1H; H-3eq), 2.06 (dd, $J_{3ax,3eq}=12.8$, $J_{3ax,4}=11.8$, 1H; H-3ax), 1.97, 1.96 (eachs, 3H; $COCH_3$), 1.94–1.80 (m, 2H; CH_2-Prop), 1.79, 1.61 (eachs, 3H; $COCH_3$), 1.05 ppm (dd, $J_{CH2,CH3}=7.8$, 3H; CH_3-Prop); ^{13}C NMR (101 MHz, C_6D_6): $\delta=137.07$ (C-*ortho*), 130.27 (C-*meta*), 129.58 (C-*para*), 88.63 (C-2), 75.99 (C-6), 71.35 (C-8), 70.27 (C-4), 68.39 (C-7), 62.88 (C-9), 52.67 ($COOCH_3$), 49.40 (C-5), 39.29 (C-3), 30.09 (C-2-Prop), 21.32, 21.07, 20.93, 20.75 (each $COCH_3$), 10.17 ppm (C-3-Prop); MALDI-TOF: *m/z*: calcd for $C_{27}H_{35}NO_{12}S$: 597.63; found: 620.3 $[M+Na]^+$.

Thiophenyl (methyl-5-*N*-butanoyl-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosylonate) (9): Compound **7** (430 mg, 734 μmol) was converted into the N-butanoyl derivative **9** by following method 2 with butanoic anhydride (240 μL, 1.47 mmol). Compound **9** was obtained as an amorphous solid. Yield: 176 mg (39%); $[\alpha]_{546}^{20}=+10$ ($c=0.5$ in $CHCl_3$); ^{1}H NMR (400 MHz, C_6D_6): $\delta=7.64$ (dd, $J_{H\text{-}m,H\text{-}o}=8.4$, $J_{H\text{-}o,H\text{-}p}=1.0$ Hz, 2H; H-o); 7.16 (dd, $J_{H\text{-}m,H\text{-}o}=8.4$, $J_{H\text{-}m,H\text{-}p}=7.6$ Hz, 2H; H-m), 7.07 (m, 1H; H-p), 5.66 (ddd, $J_{7,8}=7.1$, $J_{8,9a}=2.8$, $J_{8,9b}=5.8$ Hz, 1H; H-8), 5.51 (dd, $J_{6,7}=2.0$, $J_{7,8}=7.1$ Hz, 1H; H-7), 4.88 (ddd, $J_{3ax,4}=11.7$, $J_{3eq,4}=4.8$, $J_{4,5}=10.4$ Hz, 1H; H-4), 4.76 (dd, $J_{8,9a}=2.8$, $J_{9a,9b}=12.5$ Hz, 1H; H-9a), 4.47 (dd, $J_{8,9b}=5.8$, $J_{9a,9b}=12.5$ Hz, 1H; H-9b), 4.35 (ddd, $J_{4,5}=10.4$, $J_{5,6}=10.7$, $J_{5,NH}=10.2$ Hz, 1H; H-5), 4.16 (d, $J_{5,NH}=10.2$, 1H; NH), 4.00 (dd, $J_{5,6}=10.7$, $J_{6,7}=2.0$ Hz, 1H; H-6), 3.21 (s, 3H; $COOCH_3$), 2.99 (dd, $J_{3ax,3eq}=12.7$, $J_{3eq,4}=4.8$ Hz, 1H; H-3eq), 2.04 (dd, $J_{3ax,3eq}=12.7$, $J_{3ax,4}=11.7$ Hz, 1H; H-3ax), 1.96, 1.93, 1.78, 1.58 (eachs, 3H; $COCH_3$), 1.91–1.82 (m, 1H; CH_aH_b-But), 1.74–1.67 (m, 1H; CH_aH_b-But), 1.63–1.51 (m, 1H; CH_2-But), 0.83 ppm (dd, $J_{CH2,CH3}=7.4$, 3H; CH_3-But); ^{13}C NMR (101 MHz, C_6D_6): $\delta=172.63$ (C-1), 137.06 (C-*ortho*), 130.25 (C-*meta*), 129.58 (C-*para*), 75.86 (C-6), 71.22 (C-8), 70.12 (C-4), 68.38 (C-7), 62.85 (C-9), 52.62 ($COOCH_3$), 49.36 (C-5), 39.35 (C-3), 38.85 (C-2-But), 21.29, 21.04, 20.91, 20.75 (each $COCH_3$), 19.39 (C-3-But), 14.72 ppm (C-4-But); MALDI-TOF: *m/z*: $C_{28}H_{37}NO_{12}S$: 611.2; found: 634.4 $[M+Na]^+$, 650.4 $[M+K]^+$.

Thiophenyl (methyl-5-*N*-isobutanoyl-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosylonate) (10): Compound **7** (700 mg, 1.20 μmol) was converted into the N-isobutanoyl derivative **10** by following method 2 with isobutanoic anhydride (400 μL, 2.41 mmol). Compound **10** was obtained as an amorphous solid. Yield: 401 mg (55%); $[\alpha]_{546}^{20}=+21$ ($c=0.5$ in $CHCl_3$); ^{1}H NMR (400 MHz, C_6D_6): $\delta=7.64$ (dd, $J_{H\text{-}m,H\text{-}o}=7.6$, $J_{H\text{-}o,H\text{-}p}=1.5$ Hz, 2H; H-o), 7.15 (dd, $J_{H\text{-}m,H\text{-}o}=7.6$, $J_{H\text{-}m,H\text{-}p}=7.6$ Hz, 2H; H-m), 7.06 (m, 1H; H-p), 5.65 (ddd, $J_{7,8}=7.1$, $J_{8,9a}=2.8$, $J_{8,9b}=5.9$ Hz, 1H; H-8), 5.50 (dd, $J_{6,7}=2.0$, $J_{7,8}=7.1$ Hz, 1H; H-7), 4.90 (ddd, $J_{3ax,4}=11.7$, $J_{3eq,4}=4.8$, $J_{4,5}=10.4$ Hz, 1H; H-4), 4.75 (dd, $J_{8,9a}=2.8$, $J_{9a,9b}=12.5$ Hz, 1H; H-9a), 4.46 (dd, $J_{8,9b}=5.9$, $J_{9a,9b}=12.5$ Hz, 1H; H-9b), 4.37 (ddd, $J_{4,5}=10.4$, $J_{5,6}=10.67$, $J_{5,NH}=10.4$ Hz, 1H; H-5), 4.25 (d, $J_{5,NH}=10.4$ Hz, 1H; NH), 4.01 (dd, $J_{5,6}=10.67$, $J_{6,7}=2.0$ Hz, 1H; H-6), 3.20 (s, 3H; $COOCH_3$), 2.97 (dd, $J_{3ax,3eq}=12.5$, $J_{3eq,4}=4.8$ Hz, 1H; H-3eq), 2.06 (dd, $J_{3ax,3eq}=12.5$, $J_{3ax,4}=11.7$ Hz, 1H; H-3ax), 1.95, 1.93 (eachs, 3H; $COCH_3$), 1.92–1.81 (m, 1H; CH-But), 1.78, 1.57 (eachs, 3H; $COCH_3$), 1.14 (d, $J_{(CH,CH3a)But}=6.9$, 3H; CH_3a-But), 0.98 ppm (d, $J_{(CH,CH3b)But}=6.9$ Hz, 3H; CH_3b-But); ^{13}C NMR (101 MHz, C_6D_6): $\delta=170.00$ (C-1), 136.66 (C-*ortho*), 129.82 (C-*para*), 129.68 (C-*meta*), 100.27 (C-2), 75.52 (C-6), 70.88 (C-8), 69.67 (C-4), 67.98 (C-7), 62.46 (C-9), 52.18 ($COOCH_3$), 48.95 (C-5), 38.95 (C-3), 35.66 (CH-But), 20.84, 2.57, 20.46, 20.29 (each $COCH_3$), 19.60 (CH_3a-But), 19.13 ppm (CH_3b-But); MALDI-TOF: *m/z*: calcd for $C_{28}H_{37}NO_{12}S$: 611.66; found: 633.7 $[M+Na]^+$, 649.6 $[M+K]^+$.

Thiophenyl (methyl-5-*N*-acetoxyacetyl-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosylonate) (11): Compound **7** (500 mg, 857 μmol) was converted into the N-acetoxyacetyl derivative **11** by following method 2 with an excess of acetoxyacetyl anhydride. Compound **11** was obtained as an amorphous solid. Yield: 211 mg (38%); $[\alpha]_{546}^{20}=+13$ ($c=0.1$ in $CHCl_3$); ^{1}H NMR (400 MHz, C_6D_6): $\delta=7.62$ (dd, $J_{H\text{-}m,H\text{-}o}=8.1$, $J_{H\text{-}o,H\text{-}p}=1.3$ Hz, 2H; H-o), 7.14 (dd, $J_{H\text{-}m,H\text{-}o}=8.1$, $J_{H\text{-}m,H\text{-}p}=7.4$ Hz, 2H; H-m), 7.06 (m, 1H; H-p), 5.65 (ddd, $J_{7,8}=7.1$, $J_{8,9a}=2.5$, $J_{8,9b}=5.3$ Hz, 1H; H-8), 5.58 (dd, $J_{6,7}=2.0$, $J_{7,8}=7.1$ Hz, 1H; H-7), 5.39 (d, $J_{5,NH}=9.9$ Hz, 1H; NH), 5.12 (ddd, $J_{3ax,4}=12.0$, $J_{3eq,4}=3.6$, $J_{4,5}=10.9$ Hz, 1H; H-4), 4.73 (dd, $J_{8,9a}=2.5$, $J_{9a,9b}=12.5$ Hz, 1H; H-9a), 4.47 (dd, $J_{8,9b}=5.3$, $J_{9a,9b}=12.5$ Hz, 1H; H-9b), 4.47 (d, $J_{CH2\text{-}Gc}=15.0$ Hz, 1H; CH_2a-Gc), 4.31 (ddd, $J_{4,5}=10.9$, $J_{5,6}=10.7$, $J_{5,NH}=9.9$ Hz, 1H; H-5), 4.15 (d, $J_{CH2\text{-}Gc}=15.0$ Hz, 1H; CH_2b-Gc), 4.11 (dd, $J_{5,6}=10.7$, $J_{6,7}=2.0$ Hz, 1H; H-6), 3.14 (s, 3H; $COOCH_3$), 2.98 (dd, $J_{3ax,3eq}=12.7$, $J_{3eq,4}=3.6$ Hz, 1H; H-3_{eq}), 2.04 (dd, $J_{3ax,3eq}=12.7$, $J_{3ax,4}=12.0$ Hz, 1H; H-3_{ax}), 1.93, 1.92, 1.79, 1.73, 1.69 ppm (eachs, 3H; $COCH_3$); ^{13}C NMR (101 MHz, C_6D_6): $\delta=130.16$, 129.46, 128.59 (each C-arom), 88.53 (C-2), 75.59 (C-6), 70.96 (C-8), 69.21 (C-4), 68.41 (C-7), 63.16 (CH_2-Gc), 62.49 (C-9), 52.55 ($COOCH_3$), 50.14 (C-5), 39.24 (C-3), 21.15, 20.94, 20.80, 20.70, 20.39 ppm (each $COCH_3$); MALDI-TOF: *m/z*: calcd for $C_{28}H_{35}NO_{14}S$: 641.64; found: 664.1 $[M+Na]^+$, 680.0 $[M+K]^+$.

Methyl 5-*N*-propanoyl-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-β-D-*glycero*-D-*galacto*-2-nonulopyranosylonate (12): Thioglycoside **8** (290 mg, 485 μmol) was hydrolysed by following method 3. Compound **12** was obtained as an amorphous solid. Yield: 160 mg (65%); $[\alpha]_{546}^{20}=-5.1$ ($c=1$ in $CHCl_3$); ^{1}H NMR (400 MHz, C_6D_6): $\delta=5.72$ (dd, $J_{6,7}=2.4$, $J_{7,8}=3.8$ Hz, 1H; H-7), 5.62 (ddd, $J_{8,9a}=2.3$, $J_{8,9b}=8.1$, $J_{9a,9b}=12.2$ Hz, 1H; H-8), 5.32 (ddd, $J_{3ax,4}=10.4$, $J_{3eq,4}=6.4$, $J_{4,5}=10.4$ Hz, 1H; H-4), 5.27 (d, $J_{5,NH}=10.4$ Hz, 1H; NH), 5.08–5.05 (m, 2H; H-9a/OH), 4.60 (ddd, $J_{4,5}=10.4$, $J_{5,6}=10.5$, $J_{5,NH}=10.4$ Hz, 1H; H-5), 4.40 (dd, $J_{5,6}=10.5$, $J_{6,7}=2.4$ Hz, 1H; H-6), 4.25 (dd, $J_{8,9b}=8.1$, $J_{9a,9b}=12.2$ Hz, 1H; H-9b), 3.32 (s, 3H; $COOCH_3$), 2.27–2.21 (m, 2H; H-3ax/H-3eq), 2.01–1.84 (m, 2H; CH_2-Prop), 1.94, 1.88, 1.71, 1.62 (eachs, 3H; $COCH_3$), 1.08 ppm (dd, $J_{(CH2,CH3)Prop}=7.6$, 3H; CH_3-Prop); ^{13}C NMR (101 MHz, C_6D_6): $\delta=95.68$ (C-2), 73.53 (C-8), 72.60 (C-6), 69.71 (C-4), 69.02 (C-7), 63.53 (C-9), 53.09 ($COOCH_3$), 49.68 (C-5), 37.12 (C-3), 30.01 (C-2-Prop), 21.06, 20.91, 20.71, 20.69 (each $COCH_3$), 10.07 ppm (C-3-Prop).

Methyl 5-*N*-butanoyl-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-β-D-*glycero*-D-*galacto*-2-nonulopyranosylonate (13): Thioglycoside **9** (100 mg, 164 μmol) was hydrolysed by following method 3. Compound **13** was obtained as an amorphous solid. Yield: 46 mg (54%); $[\alpha]_{546}^{20}=-3$ ($c=0.5$ in $CHCl_3$); ^{1}H NMR (400 MHz, C_6D_6): $\delta=5.66$ (dd, $J_{6,7}=2.3$, $J_{7,8}=4.3$ Hz, 1H; H-7), 5.61 (ddd, $J_{7,8}=4.3$, $J_{8,9a}=2.0$, $J_{8,9b}=7.9$ Hz, 1H; H-8), 5.32 (m, 1H; H-4), 5.03 (dd, $J_{8,9a}=2.0$, $J_{9a,9b}=11.9$ Hz, 1H; H-9a), 4.97 (d, $J_{5,NH}=10.2$ Hz, 1H; NH), 4.58 (ddd, $J_{4,5}=10.2$, $J_{5,6}=10.6$, $J_{5,NH}=10.2$ Hz, 1H; H-5), 4.32 (dd, $J_{5,6}=10.6$, $J_{6,7}=2.3$ Hz, 1H; H-6), 4.24 (dd, $J_{8,9b}=7.9$, $J_{9a,9b}$11.9 Hz, 1H; H-9b), 3.29 (s, 3H; $COOCH_3$), 2.25–2.20 (m, 2H; H-3_{ax}/3_{eq}), 1.92, 1.87, 1.70, 1.63 (eachs, 3H; $COCH_3$), 1.92–1.79 (m, 2H; CH_2a-But), 1.65–1.58 (m, 2H; CH_2b-But), 0.83 ppm (dd, $J_{CH2,CH3}=7.6$ Hz; 3HCH_3-But); ^{13}C NMR (101 MHz, C_6D_6): $\delta=95.72$ (C-2), 73.41 (C-6), 72.53 (C-8), 69.81 (C-4), 69.00 (C-7), 63.72 (C-9), 53.24 ($COOCH_3$), 49.66 (C-5), 38.92 (C-3), 37.17 (C-2-But), 21.16, 21.04, 20.89, 20.83 (each $COCH_3$), 19.45 (C-3-But), 14.29 ppm (CH_3-But); MALDI-TOF: *m/z*: calcd for $C_{22}H_{33}NO_{13}$: 519.20; found: 542.2 $[M+Na]^+$.

Methyl 5-*N*-isobutanoyl-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-β-D-*glycero*-D-*galacto*-2-nonulopyranosylonate (14): Thioglycoside **10** (390 mg, 638 μmol) was hydrolysed by following method 3. Compound **14** was obtained as an amorphous solid. Yield: 258 mg (78%); $[\alpha]_{546}^{20}=-38$ ($c=1$ in $CHCl_3$); ^{1}H NMR (400 MHz, C_6D_6): $\delta=5.64$ (dd, $J_{6,7}=2.3$, $J_{7,8}=4.0$ Hz, 1H; H-7); 5.59 (ddd, $J_{7,8}=4.0$, $J_{8,9a}=2.3$, $J_{8,9b}=8.0$ Hz, 1H; H-8), 5.34 (ddd, $J_{3ax,4}=11.3$, $J_{3eq,4}=5.3$, $J_{4,5}=10.3$ Hz, 1H; H-4), 5.03 (d, $J_{5,NH}=10.3$ Hz, 1H; NH), 5.02 (dd, $J_{8,9a}=2.3$, $J_{9a,9b}=12.3$ Hz, 1H; H-9a), 4.90 (d, $J_{3ax,OH}=1.5$ Hz, 1H, OH), 4.57 (ddd, $J_{4,5}=10.3$, $J_{5,6}=10.5$, $J_{5,NH}=10.3$ Hz, 1H; H-5), 4.33 (dd, $J_{5,6}=10.5$, $J_{6,7}=2.3$ Hz, 1H; H-6), 4.23 (dd, $J_{8,9b}=8.0$,

$J_{9a,9b}$ = 12.3 Hz, 1H; H-9b), 3.30 (s, 3H; $COOCH_3$), 2.26 (ddd, $J_{3ax,3eq}$ = 12.8, $J_{3ax,4}$ = 11.3, $J_{3ax,OH}$ = 1.5 Hz, 1H; H-3_{ax}), 2.19 (dd, $J_{3ax,3eq}$ = 12.8, $J_{3eq,4}$ = 5.3 Hz, 1H; H-3_{eq}), 2.05–1.99 (m, 1H; CH-But), 1.92, 1.87, 1.70, 1.63 (each s, 3H; $COCH_3$), 1.17 (d, $J_{(CH,CH3a)But}$ = 6.9, 3H; CH_3a-But), 1.03 ppm (d, $J_{(CH,CH3b)But}$ = 6.9 Hz, 3H; CH_3b-But); ^{13}C NMR (101 MHz, C_6D_6): δ = 95.33 (C-2), 73.00 (C-8), 72.15 (C-6), 69.32 (C-4), 68.54 (C-7), 63.31 (C-9), 52.81 ($COOCH_3$), 49.27 (C-5), 36.79 (C-3), 35.72 (CH-But), 20.71, 20.57, 20.42, 20.38 (each $COCH_3$), 19.69 (CH_3a-But), 19.18 ppm (CH_3b-But); MALDI-TOF: *m*/*z*: calcd for $C_{22}H_{33}NO_{13}$: 519.50; found: 542.0 $[M+Na]^+$.

Methyl 5-*N*-acetoxyacetyl-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-β-D-*glycero*-D-*galacto*-2-nonulopyranosylonate (15): Thioglycoside **11** (200 mg, 312 μmol) was hydrolysed by following method 3. Compound **15** was obtained as an amorphous solid. Yield: 111 mg (65%); $[\alpha]_{546}^{20} = -20$ (c = 1 in $CHCl_3$); ^{1}H NMR (400 MHz, C_6D_6): δ = 5.83 (d, $J_{5,NH}$ = 9.9, 1H; NH), 5.64 (dd, $J_{6,7}$ = 2.0, $J_{7,8}$ = 4.8 Hz; 1HH-7), 5.59 (ddd, $J_{7,8}$ = 4.8, $J_{8,9a}$ = 2.0, $J_{8,9b}$ = 7.6, $J_{9a,9b}$ = 12.2 Hz, 1H; H-8), 5.45 (ddd, $J_{3ax,4}$ = 11.7, $J_{3eq,4}$ = 5.1, $J_{4,5}$ = 10.4 Hz; 1HH-4), 4.96 (dd, $J_{8,9a}$ = 2.0, $J_{9a,9b}$ = 12.2 Hz, 1H; H-9a), 4.90 (d, $J_{3ax,OH}$ = 2.0 Hz, 1H; OH), 4.56 (ddd, $J_{4,5}$ = 10.4, $J_{5,6}$ = 10.4, $J_{5,NH}$ = 9.9 Hz, 1H; H-5), 4.53 (d, $J_{CH2\text{-}Gc}$ = 15.0 Hz, 1H; CH_2a-Gc), 4.29 (dd, $J_{5,6}$ = 10.4, $J_{6,7}$ = 2.0 Hz, 1H; H-6), 4.23 (d, $J_{CH2\text{-}Gc}$ = 15.0 Hz, 1H; CH_2b-Gc), 4.20 (dd, $J_{8,9b}$ = 7.6, $J_{9a,9b}$ = 12.2 Hz, 1H; H-9b), 3.29 (s, 3H; $COOCH_3$), 2.26 (ddd, $J_{3ax,3eq}$ = 12.7, $J_{3ax,4}$ = 11.7, $J_{3ax,OH}$ = 2.0 Hz, 1H; H-3_{ax}), 2.15 (dd, $J_{3ax,3eq}$ = 12.7, $J_{3eq,4}$ = 5.1 Hz, 1H; H-3_{eq}), 1.90, 1.84, 1.79, 1.75, 1.69 ppm (each s, 3H; $COCH_3$); ^{13}C NMR (101 MHz, C_6D_6): δ = 95.01 (C-2), 72.13 (C-8), 71.46 (C-6), 68.44 (C-4), 68.24 (C-7), 62.95 (C-9), 62.65 (CH_2-Gc), 52.53 ($COOCH_3$), 49.68 (C-5), 36.50 (C-3), 20.39, 20.32, 20.23, 20.09, 19.88 ppm (each $COCH_3$); MALDI-TOF: *m*/*z*: calcd for $C_{22}H_{31}NO_{15}$: 549.48; found: 571.9 $[M+Na]^+$.

Methyl 5-*N*-propanoyl-4,7,8,9-tetra-*O*-acetyl-2-chloro-3,5-dideoxy-β-D-*glycero*-D-*galacto*-2-nonulopyranosylonate (16): Compound **12** (150 mg, 297 μmol) was dissolved in acetyl chloride (35 mL). Methanol (0.6 mL) was added dropwise at 0°C. The solution was stirred for 17 h and subsequently evaporated. Compound **16** was obtained as an amorphous solid. Yield: 154 mg (99%); $[\alpha]_{546}^{20} = +55$ (c = 1 in $CHCl_3$); ^{1}H NMR (400 MHz, C_6D_6): δ = 5.58 (dd, $J_{6,7}$ = 2.2, $J_{7,8}$ = 6.3 Hz, 1H; H-7), 5.53 (ddd, $J_{7,8}$ = 6.3, $J_{8,9a}$ = 2.5, $J_{8,9b}$ = 6.0 Hz, 1H; H-8), 5.29 (ddd, $J_{3ax,4}$ = 10.0, $J_{3eq,4}$ = 4.5, $J_{4,5}$ = 10.0 Hz, 1H; H-4), 4.81 (dd, $J_{8,9a}$ = 2.5, $J_{9a,9b}$ = 12.6 Hz, 1H; H-9a), 4.54 (d, $J_{5,NH}$ = 10.0 Hz, 1H; NH), 4.48 (ddd, $J_{4,5}$ = 10.0, $J_{5,6}$ = 10.0, $J_{5,NH}$ = 10.0 Hz, 1H; H-5), 4.28 (dd, $J_{8,9b}$ = 6.0, $J_{9a,9b}$ = 12.6 Hz, 1H; H-9b), 4.25 (dd, $J_{5,6}$ = 10.0, $J_{6,7}$ = 2.2 Hz, 1H; H-6), 3.36 (s, 3H; $COOCH_3$), 2.73 (dd, $J_{3ax,3eq}$ = 14.0, $J_{3eq,4}$ = 4.5 Hz, 1H; H-3eq), 2.02 (dd, $J_{3ax,3eq}$ = 14.0, $J_{3ax,4}$ = 10.0 Hz, 1H; H-3ax), 1.92, 1.91 (each s, 3H; $COCH_3$), 1.90–1.76 (m, 2H; CH_2-Prop), 1.75, 1.62 (each s, 3H; $COCH_3$), 1.07 ppm (dd, $J_{CH2,CH3}$ = 7.7, 3H; CH_3-Prop); ^{13}C NMR (101 MHz, C_6D_6): δ = 97.75 (C-2), 74.80 (C-6), 71.08 (C-8), 68.66 (C-4), 67.24 (C-7), 62.60 (C-9), 53.18 ($COOCH_3$), 48.46 (C-5), 41.15 (C-3), 29.62 (C-2-Prop), 20.74, 20.54, 20.36, 20.26 (each $COCH_3$), 9.65 ppm (C-3-Prop); MALDI-TOF: *m*/*z*: calcd for $C_{21}H_{30}ClNO_{12}$: 523.92; found: 545.9 $[M+Na]^+$.

Methyl 5-*N*-butanoyl-4,7,8,9-tetra-*O*-acetyl-2-chloro-3,5-dideoxy-β-D-*glycero*-D-*galacto*-2-nonulopyranosylonate (17): Compound **13** (251 mg, 484 μmol) was dissolved in acetyl chloride (65 mL). Methanol (1.2 mL) was added dropwise at 0°C. The solution was stirred for 17 h and subsequently evaporated. Compound **17** was obtained as an amorphous solid. Yield: 260 mg (100%); $[\alpha]_{546}^{20} = +45$ (c = 1 in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.35–5.28 (m, 2H; H-4/H-7), 5.20 (d, $J_{5,NH}$ = 9.7 Hz, 1H, NH), 5.06 (ddd, $J_{7,8}$ = 6.6, $J_{8,9a}$ = 2.8, $J_{8,9b}$ = 5.8 Hz, 1H; H-8), 4.31 (dd, $J_{8,9a}$ = 2.8, $J_{9a,9b}$ = 12.4 Hz, 1H; H-9a), 4.25 (dd, $J_{5,6}$ = 10.9, $J_{6,7}$ = 1.8 Hz, 1H; H-6), 4.12 (ddd, $J_{4,5}$ = 10.0, $J_{5,6}$ = 10.9, $J_{5,NH}$ = 9.7 Hz, 1H; H-5), 3.95 (dd, $J_{8,9b}$ = 5.8, $J_{9a,9b}$ = 12.4 Hz, 1H; H-9b), 3.77 (s, 3H; $COOCH_3$), 2.68 (dd, $J_{3ax,3eq}$ = 13.9, $J_{3eq,4}$ = 4.7 Hz, 1H; H-3_{eq}), 2.17 (dd, $J_{3ax,3eq}$ = 13.9, $J_{3ax,4}$ = 11.2 Hz, 1H; H-3_{ax}), 2.02, 1.97, 1.95, 1.93 (each s, 3H; $COCH_3$), 1.99–1.91 (m, 2H; CH_2a-But), 1.51–1.44 (m, 2H; CH_2b-But), 0.82 ppm (dd, $J_{CH2,CH3}$ = 7.4 Hz, 3H; CH_3-But); ^{13}C NMR (101 MHz, $CDCl_3$): δ = 97.00 (C-2), 74.37 (C-6), 70.35 (C-8), 69.05 (C-4), 67.28 (C-7), 62.48 (C-9), 54.19 ($COOCH_3$), 48.97 (C-5), 41.07 (C-3), 39.00 (C-2-But), 21.34, 21.26, 21.17, 21.18 (each $COCH_3$), 19.18 (C-3-But), 14.10 ppm (C-4-But).

Methyl 5-*N*-isobutanoyl-4,7,8,9-tetra-*O*-acetyl-2-chloro-3,5-dideoxy-β-D-*glycero*-D-*galacto*-2-nonulopyranosylonate (18): Compound **14** (253 mg, 487 μmol) was dissolved in acetyl chloride (30 mL). Methanol (0.6 mL) was added dropwise at 0°C. The solution was stirred for 17 h and subsequently evaporated. Compound **18** was obtained as an amorphous solid. Yield: 261 mg (100%); $[\alpha]_{546}^{20} = +8$ (c = 1 in $CHCl_3$); ^{1}H NMR (400 MHz, C_6D_6): δ = 5.56–5.51 (m, 2H; H-7/H-8), 5.29 (ddd, $J_{3ax,4}$ = 11.2, $J_{3eq,4}$ = 4.8, $J_{4,5}$ = 10.7 Hz, 1H; H-4), 4.81 (dd, $J_{8,9a}$ = 2.0, $J_{9a,9b}$ = 12.5 Hz, 1H; H-9a), 4.47 (ddd, $J_{4,5}$ = 10.7, $J_{5,6}$ = 10.7, $J_{5,NH}$ = 10.7 Hz, 1H; H-5), 4.34 (d, $J_{5,NH}$ = 10.7 Hz, 1H; NH), 4.28 (dd, $J_{8,9b}$ = 5.8, $J_{9a,9b}$ = 12.5 Hz, 1H; H-9b), 4.23 (dd, $J_{5,6}$ = 10.7, $J_{6,7}$ = 2.0 Hz, 1H; H-6), 3.35 (s, 3H; $COOCH_3$), 2.72 (dd, $J_{3ax,3eq}$ = 13.0, $J_{3eq,4}$ = 4.8 Hz, 1H; H-3_{eq}), 2.01 (dd, $J_{3ax,3eq}$ = 13.0, $J_{3ax,4}$ = 11.2 Hz, 1H; H-3_{ax}), 1.96–1.91 (m, 1H; CH-But), 1.90, 1.89, 1.74, 1.60 (each s, 3H; $COCH_3$), 1.16 (d, $J_{(CH,CH3a)But}$ = 6.8 Hz, 3H; CH_3a-But), 1.01 ppm (d, $J_{(CH,CH3b)But}$ = 7.1 Hz, 3H; CH_3b-But); ^{13}C NMR (101 MHz, C_6D_6): δ = 98.03 (C-2), 72.11 (C-6), 71.35 (C-8), 68.75 (C-4), 67.46 (C-7), 62.90 (C-9), 53.48 ($COOCH_3$), 48.57 (C-5), 41.47 (C-3), 35.90 (CH-But), 21.02, 20.83, 20.82, 20.68 (each $COCH_3$), 19.39 (CH_3a-But), 19.36 ppm (CH_3b-But); MALDI-TOF: *m*/*z*: calcd for $C_{22}H_{32}ClNO_{12}$: 537.94; found: 559.9 $[M+Na]^+$.

Methyl 5-*N*-acetoxyacetyl-4,7,8,9-tetra-*O*-acetyl-2-chloro-3,5-dideoxy-β-D-*glycero*-D-*galacto*-2-nonulopyranosylonate (19): Compound **15** (104 mg, 189 μmol) was dissolved in acetyl chloride (30 mL). Methanol (0.6 mL) was added dropwise at 0°C. The solution was stirred for 17 h and subsequently evaporated. Compound **19** was obtained as an amorphous solid. Yield: 102 mg (95%); $[\alpha]_{546}^{20} = -41$ (c = 1 in $CHCl_3$); ^{1}H NMR (400 MHz, C_6D_6): δ = 5.60 (dd, $J_{6,7}$ = 2.3, $J_{7,8}$ = 6.4 Hz, 1H; H-7), 5.53 (ddd, $J_{7,8}$ = 6.4, $J_{8,9a}$ = 2.8, $J_{8,9b}$ = 6.1 Hz, 1H; H-8), 5.38 (ddd, $J_{3ax,4}$ = 11.2, $J_{3eq,4}$ = 4.8, $J_{4,5}$ = 10.4 Hz, 1H, H-4), 5.24 (d, $J_{5,NH}$ = 10.4, 1H, NH); 4.79 (dd, $J_{8,9a}$ = 2.8, $J_{9a,9b}$ = 12.5 Hz, 1H; H-9a), 4.48 (d, $J_{CH2\text{-}Gc}$ = 14.8, 1H; CH_2a-Gc), 4.45 (ddd, $J_{4,5}$ = 10.4, $J_{5,6}$ = 10.7, $J_{5,NH}$ = 10.4 Hz, 1H; H-5), 4.26 (dd, $J_{8,9b}$ = 6.1, $J_{9a,9b}$ = 12.5 Hz, 1H; H-9b), 4.21 (dd, $J_{5,6}$ = 10.7, $J_{6,7}$ = 2.3 Hz, 1H; H-6), 4.16 (d, $J_{CH2\text{-}Gc}$ = 14.8 Hz, 1H; CH_2b-Gc), 3.34 (s, 3H; $COOCH_3$), 2.64 (dd, $J_{3ax,3eq}$ = 14.0, $J_{3eq,4}$ = 4.8 Hz, 1H; H-3_{eq}), 1.97 (dd, $J_{3ax,3eq}$ = 14.0, $J_{3ax,4}$ = 11.2 Hz, 1H; H-3_{ax}), 1.91, 1.88, 1.78, 1.76, 1.74 ppm (each s, 3H; $COCH_3$); ^{13}C NMR (101 MHz, C_6D_6): δ = 74.92 (C-6), 71.15 (C-8), 68.07 (C-4), 67.56 (C-7), 63.16 (C-9), 62.73 (CH_2-Gc), 53.46 ($COOCH_3$), 49.30 (C-5), 41.48 (C-3), 21.02, 20.90, 20.68, 20.67, 20.43 ppm (each $COCH_3$); MALDI-TOF: *m*/*z*: calcd for $C_{22}H_{30}ClNO_{14}$: 567.92; found: 589.9 $[M+Na]^+$.

4-Nitrophenyl (methyl-5-*N*-propanoyl-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosylonate) (20): Compound **16** (160 mg, 305 μmol), tetrabutylammonium hydrogen sulfate (120 mg) and 4-nitrophenol (75.0 mg, 539 μmol) were dissolved in dichloromethane (4.0 mL) and stirred vigorously with aqueous sodium hydroxide (4.0 mL, 1M) for 3 h at room temperature. After this time, the organic layer was washed with saturated aqueous sodium hydrogencarbonate, dried ($MgSO_4$) and evaporated. The residue was purified by column chromatography (ethyl acetate) on silica gel. Compound **20** was obtained as an amorphous solid. Yield: 80.0 mg (42%); $[\alpha]_{546}^{20} = +20$ (c = 1 in $CHCl_3$); ^{1}H NMR (400 MHz, C_6D_6): δ = 8.09 (d, $J_{(Ha,Hb)arom}$ = 9.2 Hz, 2H; H-a_{arom}), 7.04 (d, $J_{(Ha,Hb)arom}$ = 9.2 Hz, 2H; H-b_{arom}), 5.71 (ddd, $J_{7,8}$ = 8.6, $J_{8,9a}$ = 2.3, $J_{8,9b}$ = 5.6 Hz, 1H; H-8), 5.49 (dd, $J_{6,7}$ = 1.8, $J_{7,8}$ = 8.6 Hz, 1H; H-7), 4.94 (ddd, $J_{3ax,4}$ = 12.4, $J_{3eq,4}$ = 4.6, $J_{4,5}$ = 10.4 Hz, 1H; H-4), 4.67 (dd, $J_{5,6}$ = 10.7, $J_{6,7}$ = 1.8 Hz, 1H; H-6), 4.44 (ddd, $J_{4,5}$ = 10.4, $J_{5,6}$ = 10.7, $J_{5,NH}$ = 9.5 Hz, 1H; H-5), 4.44 (dd, $J_{8,9a}$ = 2.3, $J_{9a,9b}$ = 12.7 Hz, 1H; H-9a), 4.23 (dd, $J_{8,9b}$ = 5.6, $J_{9a,9b}$ = 12.7 Hz, 1H; H-9b), 4.21 (d, $J_{5,NH}$ = 9.5 Hz, 1H; NH), 3.09 (s, 3H; $COOCH_3$), 2.74 (dd, $J_{3ax,3eq}$ = 13.0, $J_{3eq,4}$ = 4.6 Hz, 1H; H-3_{eq}), 2.23 (dd, $J_{3ax,3eq}$ = 13.0, $J_{3ax,4}$ = 12.4 Hz, 1H; H-3_{ax}), 2.06, 1.86 (each s, 3H; $COCH_3$), 1.84–1.68 (m, 2H; CH_2-Prop), 1.77, 1.59 (each s, 3H; $COCH_3$), 1.03 ppm (dd, $J_{CH2,CH3}$ = 7.4 Hz, 3H; CH_3-Prop); ^{13}C NMR (101 MHz, C_6D_6): δ = 126.23 (C-a_{arom}), 119.20 (C-b_{arom}), 100.26 (C-2), 74.85 (C-6), 69.87 (C-8), 68.73 (C-4), 67.73 (C-7), 62.87 (C-9), 53.10 ($COOCH_3$), 49.43 (C-5), 39.65 (C-3), 30.04 (C-2-Prop), 21.23, 20.86, 20.76, 20.61 (each $COCH_3$), 10.11 ppm (CH_3-Prop); MALDI-TOF: *m*/*z*: calcd for $C_{27}H_{34}NO_{15}$: 626.56; found: 649.0 $[M+Na]^+$, 665.0 $[M+K]^+$.

4-Nitrophenyl (methyl-5-*N*-butanoyl-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosylonate) (21): Compound **17**

(260 mg, 483 μmol), tetrabutylammonium hydrogen sulfate (200 mg) and 4-nitrophenol (105 mg, 755 μmol) were dissolved in dichloromethane (2.5 mL) and stirred vigorously with aqueous sodium hydroxide (2.0 mL, 1 M) for 3 h at room temperature. After this time, the organic layer was washed with saturated aqueous sodium hydrogencarbonate, dried ($MgSO_4$) and evaporated. The residue was purified by column chromatography (ethyl acetate) on silica gel. Compound **25** was obtained as an amorphous solid. Yield: 177 mg (57%); $[\alpha]_{546}^{20}=+16$ ($c=0.5$ in $CHCl_3$); ^{1}H NMR (400 MHz, C_6D_6): $\delta=8.09$ (d, $J_{(Ha,Hb)arom}=9.4$ Hz, 2H; H-a_{arom}), 7.05 (d, $J_{(Ha,Hb)arom}=9.4$ Hz, 2H; H-b_{arom}), 5.73 (ddd, $J_{7,8}=8.6$, $J_{8,9a}=2.7$, $J_{8,9b}=5.6$ Hz, 1H, H-8), 5.50 (dd, $J_{6,7}=2.0$, $J_{7,8}=8.6$ Hz, 1H; H-7), 4.97 (ddd, $J_{3ax,4}=12.7$, $J_{3eq,4}=4.6$, $J_{4,5}=10.9$ Hz, 1H; H-4), 4.72 (dd, $J_{5,6}=10.6$, $J_{6,7}=2.0$ Hz, 1H; H-6), 4.49–4.42 (m, 2H; H-5/H-9a), 4.24 (dd, $J_{8,9b}=5.6$, $J_{9a,9b}=12.5$ Hz, 1H; H-9b), 4.20 (d, $J_{5,NH}=9.7$ Hz, 1H; NH), 3.08 (s, 3H; $COOCH_3$), 2.72 (dd, $J_{3ax,3eq}=13.2$, $J_{3eq,4}=4.6$ Hz, 1H; H-3_{eq}), 2.23 (dd, $J_{3ax,3eq}=13.2$, $J_{3ax,4}=12.7$ Hz, 1H; H-3_{ax}), 2.07, 1.85, 1.77, 1.61 (each s, 3H; $COCH_3$), 1.90–1.49 (m, 4H; 2×CH_2), 0.84 ppm (dd, $J_{CH2,CH3}=7.2$ Hz, 3H; CH_3); ^{13}C NMR (101 MHz, C_6D_6): $\delta=126.23$ (C-a_{arom}), 119.20 (C-b_{arom}), 74.86 (C-6), 69.89 (C-8), 68.72 (C-4), 67.83 (C-7), 62.92 (C-9), 53.11 ($COOCH_3$), 49.37 (C-5), 39.69 (C-3), 38.79 (C-2-But), 21.24, 20.88, 20.76, 20.69 (each $COCH_3$), 19.40 (C-3-But), 14.24 ppm (CH_3-But); MALDI-TOF: m/z: calcd for $C_{28}H_{36}N_2O_{15}$: 640.60; found: 663.3 $[M+Na]^+$, 679.4 $[M+K]^+$.

4-Nitrophenyl (methyl-5-*N*-isobutanoyl-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosylonate) (22): Compound **18** (255 mg, 474 μmol), tetrabutylammonium hydrogen sulfate (200 mg) and 4-nitrophenol (105 mg, 755 μmol) were dissolved in dichloromethane (2.8 mL) and stirred vigorously with aqueous sodium hydroxide (2.0 mL, 1 M) for 3 h at room temperature. The organic layer was washed with saturated aqueous sodium hydrogencarbonate, dried ($MgSO_4$) and evaporated. The residue was purified by column chromatography (ethyl acetate) on silica gel. Compound **22** was obtained as an amorphous solid. Yield: 133 mg (44%); $[\alpha]_{546}^{20}=+16$ ($c=1$ in $CHCl_3$); ^{1}H NMR (400 MHz, C_6D_6): $\delta=8.01$ (d, $J_{(Ha,Hb)arom}=9.2$ Hz, 2H; H-a_{arom}), 7.04 (d, $J_{(Ha,Hb)arom}=9.2$ Hz, 2H; H-b_{arom}), 5.72 (ddd, $J_{7,8}=8.7$, $J_{8,9a}=2.8$, $J_{8,9b}=5.9$ Hz, 1H; H-8), 5.49 (dd, $J_{6,7}=2.0$, $J_{7,8}=8.7$ Hz, 1H; H-7), 4.98 (ddd, $J_{3ax,4}=12.7$, $J_{3eq,4}=4.6$, $J_{4,5}=10.2$ Hz, 1H; H-4), 4.72 (dd, $J_{5,6}=10.2$, $J_{6,7}=2.0$ Hz, 1H; H-6), 4.46 (ddd, $J_{4,5}=10.2$, $J_{5,6}=10.2$, $J_{5,NH}=10.9$ Hz, 1H; H-5), 4.43 (dd, $J_{8,9a}=2.8$, $J_{9a,9b}=12.5$ Hz, 1H; H-9a), 4.25 (d, $J_{5,NH}=10.9$ Hz, 1H; NH), 4.23 (dd, $J_{8,9b}=5.9$, $J_{9a,9b}=12.5$ Hz, 1H; H-9b), 3.08 (s, 3H; $COOCH_3$), 2.73 (dd, $J_{3ax,3eq}=12.7$, $J_{3eq,4}=4.6$ Hz, 1H; H-3_{eq}), 2.24 (dd, $J_{3ax,3eq}=12.7$, $J_{3ax,4}=12.7$ Hz, 1H; H-3_{ax}), 2.06 (s, 3H; $COCH_3$), 1.95–1.91 (m, 1H; CH-But), 1.85, 1.77, 1.60 (each s, 3H; $COCH_3$), 1.13 (d, $J_{(CH,CH3a)But}=6.9$ Hz, 3H; CH_3a-But), 0.97 ppm (d, $J_{(CH,CH3b)But}=6.9$ Hz, 3H; CH_3b-But); ^{13}C NMR (101 MHz, C_6D_6): $\delta=118.88$ (C-a_{arom}), 115.37 (C-b_{arom}), 74.48 (C-6), 69.52 (C-8), 68.25 (C-4), 67.41 (C-7), 62.52 (C-9), 52.67 ($COOCH_3$), 48.95 (C-5), 39.26 (C-3), 38.79 (CH-But), 20.80, 20.41, 20.31, 20.23 (each $COCH_3$), 19.62 (CH_3a-But), 19.08 ppm (CH_3b-But); MALDI-TOF: m/z: calcd for $C_{28}H_{36}N_2O_{15}$: 640.59; found: 663.0 $[M+Na]^+$.

4-Nitrophenyl (methyl-5-*N*-acetoxyacetyl-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosylonate) (23): Compound **19** (92 mg, 192 μmol), tetrabutylammonium hydrogen sulfate (69 mg) and 4-nitrophenol (38.0 mg, 273 μmol) were dissolved in dichloromethane (1.0 mL) and stirred vigorously with aqueous sodium hydroxide (0.7 mL, 1 M) for 3 h at room temperature. After this time, the organic layer was washed with saturated aqueous sodium hydrogencarbonate, dried ($MgSO_4$) and evaporated. The residue was purified by column chromatography (ethyl acetate) on silica gel. Compound **23** was obtained as an amorphous solid. Yield: 61 mg (47%); $[\alpha]_{546}^{20}=+21$ ($c=1$ in $CHCl_3$); ^{1}H NMR (400 MHz, C_6D_6): $\delta=8.09$ (d, $J_{(Ha,Hb)arom}=9.4$ Hz, 2H; H-a_{arom}), 7.04 (d, $J_{(Ha,Hb)arom}=9.4$ Hz, 2H; H-b_{arom}), 5.72 (ddd, $J_{7,8}=8.6$, $J_{8,9a}=3.0$, $J_{8,9b}=5.3$ Hz, 1H; H-8), 5.55 (dd, $J_{6,7}=1.8$, $J_{7,8}=8.6$ Hz, 1H; H-7), 5.37 (d, $J_{5,NH}=10.2$ Hz, 1H; NH), 5.24 (ddd, $J_{3ax,4}=12.5$, $J_{3eq,4}=4.6$, $J_{4,5}=10.7$ Hz, 1H; H-4), 4.84 (dd, $J_{5,6}=10.7$, $J_{6,7}=1.8$ Hz, 1H; H-6), 4.42 (d, $J_{CH2\text{-}Gc}=14.7$ Hz, 1H; CH_2a-Gc), 4.42 (dd, $J_{8,9a}=3.0$, $J_{9a,9b}=12.5$ Hz, 1H; H-9a), 4.34 (ddd, $J_{4,5}=10.7$, $J_{5,6}=10.7$, $J_{5,NH}=10.2$ Hz, 1H; H-5), 4.24 (dd, $J_{8,9b}=5.3$, $J_{9a,9b}=12.5$ Hz, 1H; H-9b), 4.11 (d, $J_{CH2\text{-}Gc}=14.7$ Hz, 1H; CH_2b-Gc), 3.04 (s, 3H; $COOCH_3$), 2.75 (dd, $J_{3ax,3eq}=13.0$, $J_{3eq,4}=4.6$ Hz, 1H; H-3_{eq}), 2.22 (dd, $J_{3ax,3eq}=13.0$, $J_{3ax,4}=12.5$ Hz, 1H; H-3_{ax}), 2.02, 1.83, 1.79, 1.78, 1.69 ppm (each s, 3H; $COCH_3$); ^{13}C NMR (101 MHz, C_6D_6): $\delta=125.79$ (C-a_{arom}), 118.85 (C-b_{arom}), 74.07 (C-6), 69.24 (C-8), 67.63 (C-7), 67.40 (C-4), 62.83 (CH_2-Gc), 62.21 (C-9), 52.70 ($COOCH_3$), 49.96 (C-5), 39.33 (C-3), 20.78, 20.45, 20.35, 20.03 ppm (each $COCH_3$); MALDI-TOF: m/z: calcd for $C_{28}H_{34}N_2O_{17}$: 670.57; found: 693.0 $[M+Na]^+$, 709.0 $[M+K]^+$.

4-Nitrophenyl (methyl-5-*N*-propanoyl-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosylonate) (24): Compound **20** (80 mg, 128 μmol) was dissolved in methanolic sodium methoxide solution (15 mL, 0.1 M) and stirred for 1.5 h at room temperature. After this time, the solution was neutralized with Amberlite IR 120 (H^+) resin and lyophilized. The residue was purified by column chromatography (ethyl acetate/methanol) on silica gel. Compound **24** was obtained as a colourless solid. Yield: 40 mg (68%); m.p. 103 °C; $[\alpha]_{546}^{20}=+33$ ($c=1$ in MeOH); ^{1}H NMR (400 MHz, MeOD): $\delta=8.20$ (d, $J_{(Ha,Hb)arom}=9.4$ Hz, 2H; H-a_{arom}), 7.34 (d, $J_{(Ha,Hb)arom}=9.4$, 2H; H-b_{arom}), 4.17 (dd, $J_{5,6}=10.4$, $J_{6,7}=1.5$ Hz, 1H, H-6), 3.90 (dd, $J_{4,5}=10.4$, $J_{5,6}=10.4$ Hz, 1H; H-5), 3.83–3.74 (m, 3H; H-4/H-8/H-9a), 3.72 (s, 3H; $COOCH_3$), 3.62 (dd, $J_{8,9b}=5.9$, $J_{9a,9b}=11.7$ Hz, 1H; H-9b), 3.51 (dd, $J_{6,7}=1.5$, $J_{7,8}=8.9$ Hz, 1H; H-7), 2.78 (dd, $J_{3ax,3eq}=12.7$, $J_{3eq,4}=4.3$ Hz, 1H; H-3_{eq}), 2.29 (ddd, $J_{(CH2,CH3)}=7.6$ Hz, 2H; CH_2-Prop), 2.04 (dd, $J_{3ax,3eq}=12.7$, $J_{3ax,4}=13.0$ Hz, 1H; H-3_{ax}), 1.15 ppm (dd, $J_{(CH2,CH3)}=7.6$ Hz, 3H, CH_3); ^{13}C NMR (101 MHz, MeOD): $\delta=126.71$ (C-a_{arom}), 121.67 (C-b_{arom}), 102.34 (C-2), 76.70 (C-6), 72.33 (C-8), 70.56 (C-7), 68.23 (C-4), 65.54 (C-9), 53.91 ($COOCH_3$), 53.57 (C-5), 42.65 (C-3), 30.59 (C-2-Prop), 10.71 ppm (CH_3-Prop); MALDI-TOF: m/z: calcd for $C_{19}H_{26}N_2O_{11}$: 458.42; found: 481.3 $[M+K]^+$.

4-Nitrophenyl (methyl-5-*N*-butanoyl-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosylonate) (25): Compound **21** (170 mg, 265 μmol) was dissolved in methanolic sodium methoxide solution (15 mL, 0.1 M) and stirred for 1.5 h at room temperature. After this time, the solution was neutralized with Amberlite IR 120 (H^+) resin and lyophilized. The residue was purified by column chromatography (ethyl acetate/methanol) on silica gel. Compound **25** was obtained as a colourless solid. Yield: 103 mg (82%), m.p. 95 °C; $[\alpha]_{546}^{20}=+60$ ($c=1$ in MeOH); ^{1}H NMR (400 MHz, MeOD): $\delta=8.21$ (d, $J_{(Ha,Hb)arom}=9.2$ Hz, 2H; H-a_{arom}), 7.35 (d, $J_{(Ha,Hb)arom}=9.2$ Hz, 2H; H-b_{arom}), 4.17 (dd, $J_{5,6}=10.4$, $J_{6,7}=1.3$ Hz, 1H; H-6), 3.92 (dd, $J_{4,5}=10.4$, $J_{5,6}=10.4$ Hz, 1H; H-5), 3.84–3.77 (m, 3H; H-4/H-8/H-9a), 3.74 (s, 3H; $COOCH_3$), 3.63 (dd, $J_{8,9b}=5.6$, $J_{9a,9b}=11.5$ Hz, 1H; H-9b), 3.54 (dd, $J_{6,7}=1.3$, $J_{7,8}=8.5$ Hz, 1H; H-7), 2.79 (dd, $J_{3ax,3eq}=12.5$, $J_{3eq,4}=4.5$ Hz, 1H; H-3_{eq}), 2.28–2.20 (m, 2H; CH_2-a-But), 2.05 (dd, $^2J_{3ax,3eq}=12.5$ Hz, $J_{3ax,4}=12.2$ Hz, 1H; H-3_{ax}), 1.72–1.62 (m, 2H; CH_2b-But), 0.98 ppm (dd, $J_{CH2,CH3}=7.4$ Hz, 3H; CH_3); ^{13}C NMR (101 MHz, MeOD): $\delta=125.18$ (C-a_{arom}), 120.17 (C-b_{arom}), 75.19 (C-6), 70.81 (C-8), 69.12 (C-7), 66.70 (C-4), 64.07 (C-9), 52.38 ($COOCH_3$), 52.31 (C-5), 41.19 (C-3), 37.93 (C-2-But), 19.18 (C-3-But), 12.96 ppm (CH_3-But); MALDI-TOF: m/z: calcd for $C_{20}H_{28}N_2O_{11}$: 472.44; found: 693.0 $[M+Na]^+$, 709.0 $[M+K]^+$.

4-Nitrophenyl (methyl-5-*N*-isobutanoyl-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosylonate) (26): Compound **22** (118 mg, 184 μmol) was dissolved in methanolic sodium methoxide solution (8 mL, 0.1 M) and stirred for 2 h at room temperature. After this time, the solution was neutralized with Amberlite IR 120 (H^+) resin and lyophilized. The residue was purified by column chromatography (ethyl acetate/methanol) on silica gel. Compound **26** was obtained as a colourless solid. Yield: 56.3 mg (65%); m.p. 115 °C; $[\alpha]_{546}^{20}=+32$ ($c=1$ in MeOH); ^{1}H NMR (400 MHz, D_2O): $\delta=8.21$ (d, $J_{(Ha,Hb)arom}=9.4$ Hz, 2H; H-a_{arom}), 7.28 (d, $J_{(Ha,Hb)arom}=9.4$ Hz, 2H; H-b_{arom}), 4.17 (dd, $J_{5,6}=10.4$, $J_{6,7}=1.5$ Hz, 1H; H-6), 3.94 (dd, $J_{4,5}=10.5$, $J_{5,6}=10.4$ Hz, 1H; H-5), 3.86–3.80 (m, 3H; H-4/H-8/H-9a), 3.62 (dd, $J_{8,9b}=6.6$, $J_{9a,9b}=12.5$ Hz, 1H; H-9b), 3.53 (dd, $J_{6,7}=1.5$, $J_{7,8}=8.9$ Hz, 1H; H-7), 2.84 (dd, $J_{3ax,3eq}=12.8$, $J_{3ax,3eq}=12.8$ Hz, 1H; H-3_{eq}), 2.55 (m, 1H; CH-But), 2.02 (dd, $J_{3ax,4}=11.7$, $J_{3ax,3eq}=12.8$ Hz, 1H; H-3_{ax}), 1.12 (d, $J_{(CH,CH3a)But}=7.1$ Hz, 3H; CH_3a-But), 1.11 ppm (d, $J_{(CH,CH3b)But}=7.1$ Hz, 3H; CH_3b-But); ^{13}C NMR (101 MHz, D_2O): $\delta=125.91$ (C-a_{arom}), 120.26 (C-b_{arom}), 102.68 (C-2), 74.14 (C-6), 71.73 (C-4), 68.70 (C-7), 67.78 (C-8), 63.10 (C-9), 51.81 (C-5), 41.35 (C-3), 35.58 (CH-But), 19.32 (CH_3a-But), 18.79 ppm (CH_3b-But); MALDI-TOF: m/z: calcd for $C_{20}H_{28}N_2O_{11}$: 472.44; found: 495.2 $[M+Na]^+$, 511.1 $[M+K]^+$.

4-Nitrophenyl (methyl-5-*N*-glycolyl-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosylonate) (27): Compound **23** (55 mg, 82 μmol) was dissolved in a methanolic sodium methoxide solution (5 mL, 0.1 M) and stirred for 2 h at room temperature. After this time, the solution was neutralized with Amberlite IR 120 (H^+) resin and lyophilized. The residue was purified by column chromatography (ethyl acetate/methanol) on silica gel. Compound **27** was obtained as a colourless solid. Yield: 28 mg (74%); m.p. 114°C; $[\alpha]_{546}^{20}=+46$ ($c=1$ in MeOH); ^{1}H NMR (400 MHz, MeOD): $\delta=8.20$ (d, $J_{(Ha,Hb)arom}=9.3$ Hz, 2H; H-a_{arom}), 7.35 (d, $J_{(Ha,Hb)arom}=9.3$ Hz, 2H; H-b_{arom}), 4.24 (dd, $J_{5,6}=10.2$, $J_{6,7}=1.5$ Hz, 1H; H-6), 4.05 (s, 2H; CH_2-Gc), 3.97 (dd, $J_{4,5}=10.2$, $J_{5,6}=10.2$ Hz, 1H; H-5), 3.90 (ddd, $J_{3ax,4}=11.5$, $J_{3eq,4}=4.6$, $J_{4,5}=10.2$ Hz, 1H; H-4), 3.82 (dd, $J_{8,9a}=2.8$, $J_{9a,9b}=11.2$ Hz, 1H; H-9a), 3.78 (ddd, $J_{7,8}=9.2$, $J_{8,9a}=2.8$, $J_{8,9b}=5.3$ Hz, 1H; H-8), 3.73 (s, 3H; $COOCH_3$), 3.62 (dd, $J_{8,9b}=5.3$, $J_{9a,9b}=11.2$ Hz, 1H; H-9b), 3.54 (dd, $J_{6,7}=1.5$, $J_{7,8}=9.2$ Hz, 1H; H-7), 2.81 (dd, $J_{3ax,3eq}=13.0$, $J_{3eq,4}=4.6$ Hz, 1H; H-3_{eq}), 2.05 ppm (dd, $J_{3ax,3eq}=13.0$, $J_{3ax,4}=11.5$ Hz, 1H; H-3_{ax}); ^{13}C NMR (101 MHz, MeOD): $\delta=125.14$ (C-a_{arom}), 120.33 (C-b_{arom}), 74.79 (C-6), 70.95 (C-8), 68.94 (C-7), 66.47 (C-4), 63.97 (CH_2-Gc), 61.51 (C-9), 52.42 ($COOCH_3$), 52.15 (C-5), 41.06 ppm (C-3); MALDI-TOF: m/z: calcd for $C_{18}H_{24}N_2O_{12}$: 460.39; found: 484.0 $[M+Na]^+$, 499.9.0 $[M+K]^+$.

Methyl (5-acetamido-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosylonic acid)-(2→3)-(β-D-galactopyranosyl)-(1→6)-α-D-glucopyranoside (29): Following method 1, donor **1** was incubated with the allolactoside **28** and TcTS for 17 h, yielding compound **29** as a colourless solid. Yield: 14 mg (87%), m.p. 142°C; $[\alpha]_{546}^{20}=+33$ ($c=1$ in H_2O); ^{1}H NMR (400 MHz, D_2O): $\delta=4.85$ (d, $J_{1,2}=1.8$ Hz, 1H; H-1); 4.54 (d, $J_{1',2'}=8.0$ Hz, 1H; H-1′), 4.22 (dd, $J_{5,6a}=2.0$; $J_{6a,6b}=11.8$ Hz, 1H; H-6a), 4.14 (dd, $J_{2',3'}=9.8$, $J_{3',4'}=3.3$ Hz, 1H; H-3′), 4.00 (m, 17H; H-2/H-2′/H-3/H-4/H-4′/H-4″/H-5/H-5′/H-5″/H-6b/H-6′a/H-6′b/H-6″/H-7″/H-8″/H-9″a/H-9″b), 3.47 (s, 3H; OCH_3), 2.81 (dd, $J_{3''ax,3''eq}=12.3$, $J_{3''eq,4''}=4.5$ Hz, 1H; H-3″eq), 2.08 (s, 3H; $COCH_3$), 1.84 ppm (dd, $J_{3''ax,4''}=12.1$, $J_{3''ax,3''eq}=12.3$ Hz, 1H; H-3″ax); ^{13}C NMR (101 MHz, D_2O): $\delta=103.39$ (C-1′), 100.10 (C-2″), 99.76 (C-1), 76.17 (C-3′), 75.29 (C-7″), 73.25, 73.23 (C-5′/C-6″), 72.15 (C-5), 71.49, 70.94, 69.52 (C-2/C-2′/C-8″), 69.45 (C-4), 68.69 (C-3), 68.56 (C-6), 68.41 (C-4″), 67.86 (C-4′), 62.91 (C-6′), 61.36 (C-9″), 55.63 (OCH_3), 52.03 (C-5″), 40.09 (C-3″), 22.40 ppm ($COCH_3$); MALDI-TOF: m/z: calcd for $C_{24}H_{41}NO_{19}$: 647.58; found: 670.4 $[M+Na]^+$, 686.3 $[M+K]^+$.

Methyl (5-acetamido-9-*O*-butanoyl-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosylonic acid)-(2→3)-(β-D-galactopyranosyl)-(1→6)-α-D-glucopyranoside (30): Following method 1, donor **2** was incubated with the allolactoside **28** and TcTS for 17 h, yielding compound **30** as a colourless solid. Yield: 14 mg (78%); m.p. 112°C; $[\alpha]_{546}^{20}=+11$ ($c=1$ in H_2O); ^{1}H NMR (400 MHz, D_2O): $\delta=4.80$ (d, $J_{1,2}=3.6$, 1H; H-1), 4.49 (d, $J_{1',2'}=8.1$ Hz, 1H; H-1′), 4.39 (dd, $J_{8'',9''a}=2.3$, $J_{9''a,9''b}=11.7$ Hz, 1H; H-9″a), 4.25 (dd, $J_{8'',9''b}=5.6$, $J_{9''a,9''b}=11.7$ Hz, 1H; H-9″b), 4.18 (dd, $J_{5,6a}=2.0$, $J_{6a,6b}=11.4$ Hz, 1H; H-6a), 4.09 (dd, $J_{2',3'}=9.9$, $J_{3',4'}=3.1$ Hz, 1H; H-3′), 4.05 (ddd, $J_{7'',8''}=5.6$, $J_{8'',9''a}=2.3$, $J_{8'',9''b}=5.6$ Hz, 1H; H-8″), 3.94–3.63 (m, 11H; H-3/H-4′/H-4″/H-5/H-5′/H-5″/H-6b/H-6′a/H-6′b/H-6″/H-7″), 3.60–3.55 (m, 2H; H-2/H-2″), 3.52 (dd, $J_{3,4}=9.4$, $J_{4,5}=9.4$ Hz, 1H; H-4), 3.42 (s, 3H; OCH_3), 2.76 (dd, $J_{3''ax,3''eq}=12.5$, $J_{3''eq,4''}=4.6$ Hz, 1H; H-3″eq), 2.41 (dd, $J_{CH2,CH2}=7.4$, 2H; CH_2(2)-But), 2.03 (s, 3H; $COCH_3$), 1.79 (dd, $J_{3''ax,3''eq}=12.5$, $J_{3''ax,4''}=12.2$ Hz, 1H; H-3″ax), 1.69 (sext, $J_{CH2,CH2}=7.4$, $J_{CH2,CH3}=7.6$ Hz, 2H; CH_2(3)-But), 0.92 ppm (dd, $J_{CH2,CH3}=7.6$ Hz, 3H; CH_3(4)-But); ^{13}C NMR (101 MHz, D_2O): $\delta=103.41$ (C-1′), 100.09 (C-2″), 99.76 (C-1), 76.18 (C-3′), 75.32 (C-7″), 73.25 (C-5′), 73.01 (C-6″), 71.52 (C-5), 70.97, 69.78, 69.78 (C-2/C-2′/C-8″), 69.51 (C-4), 68.67 (C-3), 68.60 (C-6), 68.37 (C-4″), 67.80 (C-4′), 65.70 (C-9″), 61.38 (C-6′), 55.64 (OCH_3), 52.04 (C-5″), 40.16 (C-3″), 36.11 (C-2-But), 22.39 ($COCH_3$), 18.73 (C-3-But), 13.21 ppm (C-4-But); MALDI-TOF: m/z: calcd for $C_{28}H_{47}NO_{20}$: 717.67; found: 740.2 $[M+Na]^+$.

Methyl (5-*N*-propanoyl-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosylonic acid)-(2→3)-(β-D-galactopyranosyl)-(1→6)-α-D-glucopyranoside (31): Following method 1, donor **3** was incubated with the allolactoside **28** and TcTS for 17 h, yielding compound **31** as a colourless solid. Yield: 7.4 mg (32%); m.p. 103°C; $[\alpha]_{546}^{20}=+16$ ($c=1$ in H_2O); ^{1}H NMR (400 MHz, D_2O): $\delta=4.82$ (d, $J_{1,2}=3.8$, 1H; H-1), 4.52 (d, $J_{1',2'}=8.0$ Hz, 1H; H-1′), 4.20 (dd, $J_{5,6a}=2.0$, $J_{6a,6b}=9.8$ Hz, 1H; H-6a), 4.12 (dd, $J_{2',3'}=11.8$, $J_{3',4'}=3.3$ Hz, 1H; H-3′), 3.97–3.53 (m, 17H; H-2/H-2′/H-3/H-4/H-4′/H-4″/H-5/H-5′/H-5″/H-6b/H-6′a/H-6′b/H-6″/H-7″/H-8″/H-9″a/H-9″b), 3.45 (s, 3H; OCH_3), 2.79 (dd, $J_{3''ax,3''eq}=12.3$, $J_{3''eq,4''}=4.5$ Hz, 1H; H-3″eq), 2.32 (ddd, $J_{CH2,CH3}=7.8$ Hz, 2H; CH_2-Prop), 1.82 (dd, $J_{3''ax,4''}=12.1$, $J_{3''ax,3''eq}=12.5$ Hz, 1H; H-3″ax), 1.13 ppm (dd, $J_{CH2,CH3}=7.8$ Hz, 3H; CH_3-Prop); ^{13}C NMR (101 MHz, D_2O): $\delta=103.38$ (C-1′), 99.75 (C-1), 76.16 (C-3′), 75.28 (C-7″), 73.24 (C-5′), 73.24 (C-6″), 73.12 (C-5), 71.48, 70.93, 70.63 (C-2/C-2′/C-8″), 69.52, 69.45 (C-4/C-3), 68.55 (C-6), 68.43 (C-4″), 67.85 (C-4′), 62.89 (C-9″), 61.34 (C-6′), 55.62 (OCH_3), 51.88 (C-5″), 40.13 (C-3″), 29.59 (CH_2-Prop), 9.85 ppm (CH_3-Prop); MALDI-TOF: m/z: calcd for $C_{25}H_{43}NO_{19}$: 661.60; found: 684.1 $[M+Na]^+$.

Methyl (5-*N*-glycolyl-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosylonic acid)-(2→3)-(β-D-galactopyranosyl)-(1→6)-α-D-glucopyranoside (34): Following method 1, donor **6** was incubated with the allolactoside **28** and TcTS for 17 h, yielding compound **34** as a colourless solid. Yield: 10 mg (60%); m.p. 139°C; $[\alpha]_{546}^{20}=+31$ ($c=1$ in H_2O); ^{1}H NMR (400 MHz, D_2O): $\delta=4.74$ (d, $J_{1,2}=3.8$ Hz, 1H; H-1), 4.44 (d, $J_{1',2'}=7.9$ Hz, 1H; H-1′), 4.12 (dd, $J_{5,6a}=1.8$, $J_{6a,6b}=11.4$ Hz, 1H; H-6a), 4.07–3.44 (m, 18H; H-2/H-2′/H-3/H-3′/H-4/H-4′/H-4″/H-5/H-5′/H-5″/H-6b/H-6′a/H-6′b/H-6″/H-7″/H-8″/H-9″a/H-9″b), 3.36 (s, 3H; OCH_3), 2.72 (dd, $J_{3''ax,3''eq}=12.5$, $J_{3''eq,4''}=4.6$ Hz, 1H; H-3″eq), 1.74 ppm (dd, $J_{3''ax,4''}=12.1$, $J_{3''ax,3''eq}=12.5$ Hz, 1H; H-3″ax); ^{13}C NMR (101 MHz, D_2O): $\delta=103.41$ (C-1′), 100.16 (C-2″), 99.77 (C-1), 76.19 (C-3′), 75.32 (C-7″), 73.27 (C-5′), 72.96 (C-6″), 72.42 (C-5), 71.51, 70.96, 69.55 (C-2/C-2′/C-8″), 69.50 (C-4), 68.58 (C-6), 68.45 (C-3), 68.34 (C-4″), 67.86 (C-4′), 62.89 (C-9″), 61.38 (CH_2-Gc), 61.37 (C-6′), 55.65 (OCH_3), 51.76 (C-5″), 40.18 ppm (C-3″); MALDI-TOF: m/z: calcd for $C_{24}H_{41}NO_{20}$: 663.58; found: 686.4 $[M+Na]^+$.

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