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Studies on the α-(1→4)- and α-(1→8)-fucosylation of sialic acid for the total assembly of the glycan portions of complex HPG-series gangliosides ☆

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Abstract—A synthetic study on α-(1→4) and α-(1→8)-fucosylation of sialic acid is reported, with the ultimate aim being the total assembly of the glycan portion of HPG-series gangliosides. In both types of fucosylations, the combination of a phenylthio fucosyl donor and a 1,5-lactamized acceptor provided high-yielding glycosylations to afford α-fucosyl-sialic acid sequences. The obtained α-Fuc*p*-(1→8)-Neu*p*NAc glycan having a 1,5-lactam bridge has been successfully transformed into the corresponding glycosyl donor.

Keywords: Fucosylation; 1,5-Lactamized sialyl acceptor; Ganglioside HPG-1; Ganglioside HPG-7

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1. Introduction

The broad diversity of sialic acid structures has been demonstrated by numerous studies in the field of natural product chemistry.[1] For example, N-glycosylation, de-N-acetylation, O-methylation, O-sulfonylation, and O-acetylation are representative modifications of sialic acids. In addition to these structural modifications, sialic acid often condenses with other sialic acid residues, forming homo-oligomers and polymers. Presumably, the structural polymorphism of sialic acid relates to the complex biological functions of sialo-oligosaccharides in cells.

To clarify the molecular basis underlying the biological roles of these various structural motifs, we have engaged in a long-term project on the synthesis of sialoglycoconjugates. In particular, our recent focus has turned to the synthesis of oligosaccharides containing intricate sialic acid congeners. We thus have previously reported the synthesis of 1,5-lactamized-[2] and 5-amino[3]-sialyl Lewis X homologues, and very recently, the first synthesis of the glycan portions of HLG-2 and Hp-s6 gangliosides,[4] which have partially modified disialic acid residues in their glycan skeletons. In the latest synthesis, we developed highly reactive 1,5-lactamized sialyl acceptors, which underwent α-(2→4)- and α-(2→8)-sialylation in good yield. In this paper, we report the successful use of 1,5-lactamized sialyl units in the production of α-(1→4)- and α-(1→8)-fucosyl sialic acid sequences, which are substructure of complex gangliosides HPG-7 and HPG-1, respectively.

2. Results and discussion

Recently, Higuchi's group identified the novel gangliosides HPG-1[5] and HPG-7[6] from the sea cucumber

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☆ Part 140 of the series: synthetic studies on sialoglycoconjugates. For Part 139 see Ref. 4.

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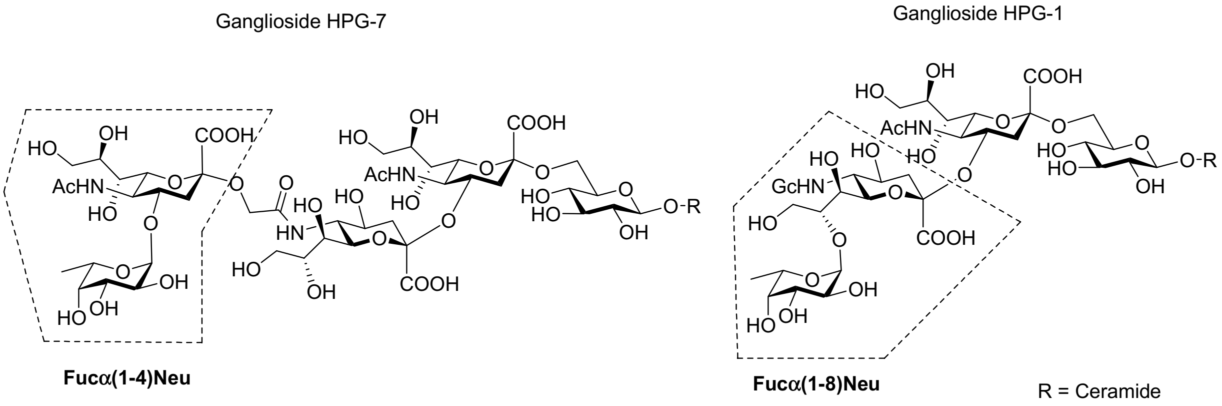


Ganglioside HPG-7

Ganglioside HPG-1

Fucα(1-4)Neu

Fucα(1-8)Neu

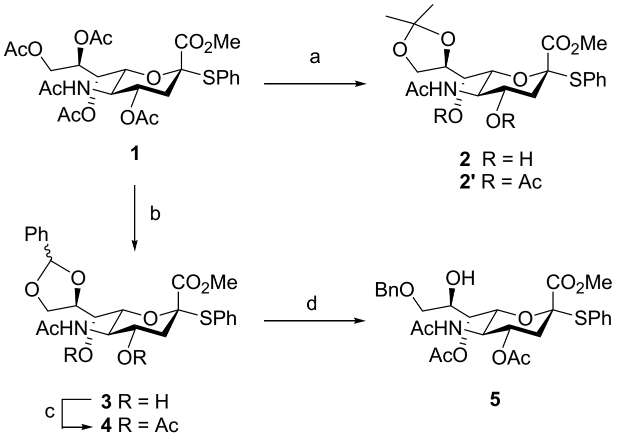
R = Ceramide

Figure 1. Structures of the HPG-7 and HPG-1 gangliosides.

Holothuria pervicax. These compounds showed neuritogenic activity toward rat pheochromocytoma PC-12 cells and therefore are attracting considerable attention with respect to the development of new compounds for treating neural disease. As shown in Figure 1, the sialic acid termini of HPG-7 and HPG-1 are glycosylated with fucopyranosyl residues through either α-(1→4) or α-(1→8) linkages. Previously, we developed a method to assemble the α-Neu*p*Gc-(2→4)-α-Neu*p*5Ac-(2→6)-Glc*p* sequence based on the combined use of a reactive 1,5-lactamized sialyl acceptor and *N*-(2,2,2-trichloroethoxycarbonyl)-protected sialyl donors.[4] To complete the synthesis of HPG-7 and HPG-1, the construction of α-Fuc*p*-(1→8)-Neu*p* and α-Fuc*p*-(1→4)-Neu*p* sequences remains to be achieved.

2.1. Design and synthesis of acceptors

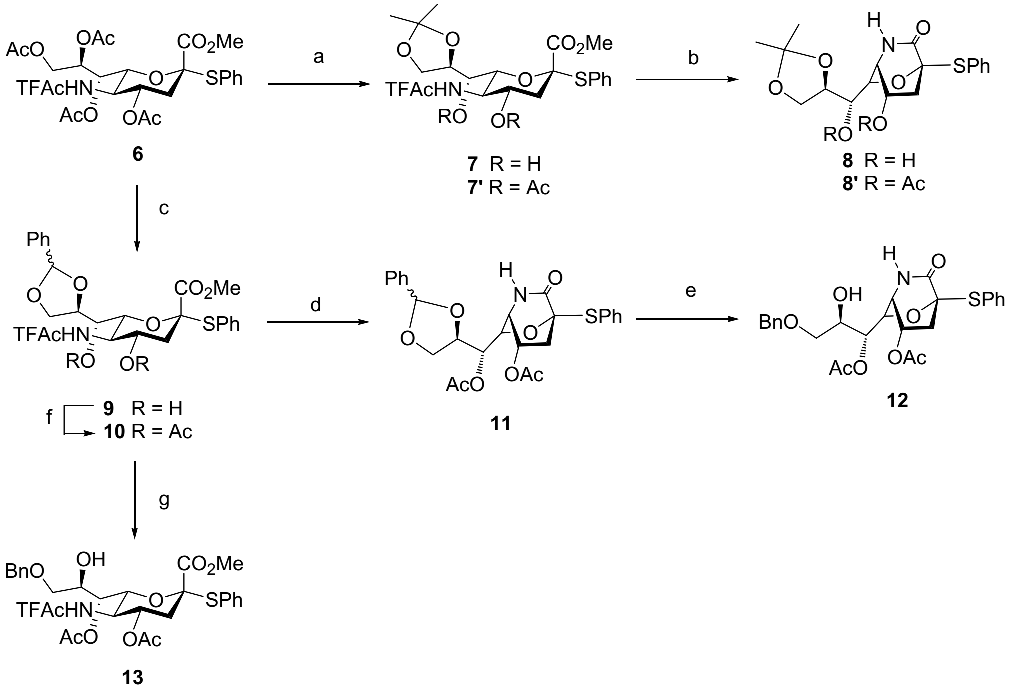
For the synthesis of the α-Fuc*p*-(1→4)-Neu*p*5Ac substructure, we designed two acceptors, the 4,7-diol sialyl thioglycoside **2** and the 1,5-lactamized sialyl thioglycoside **8**. For the α-Fuc*p*-(1→8)-Neu*p*5Gc sequence, 1,5-lactamized and *N*-trifluoroacetyl (NTFA)-protected sialyl acceptors, **12** and **13**, were chosen. In addition, the N-acetylated (NAc) sialyl acceptor **5** was used to evaluate the effect of the C5 substituent on the reactivity of the C8 hydroxyl group.



Scheme 1. Preparation of NAc sialyl acceptors. Reagents and conditions: (a) (i) $NaOCH_3$, CH_3OH, rt, 27 h; (ii) 2,2-dimethoxypropane, CSA/CH_3CN, rt, 1.5 h, 60% (two steps); (b) (i) $NaOCH_3$, CH_3OH, rt, 27 h; (ii) $PhCH(OCH_3)_2$, CSA/DMF, 40 °C, 18 h, 77% (two steps); (c) Ac_2O, pyridine, rt, quant.; (d) $BH_3{\cdot}N(CH_3)_2$, $AlCl_3$/THF, 4 Å molecular sieves, 5 h, 0 °C→45 °C, 49%.

From the known sialic acid phenyl thioglycoside, **1**,[7] straightforward chemistry furnished acceptors **2** and **5** (Scheme 1). Thus, treatment of **1** with sodium methoxide followed by dimethoxypropane and camphorsulfonic acid yielded **2** in 60% overall yield, while deacetylation of **1** followed by reaction with benzaldehyde dimethyl acetal and camphorsulfonic acid gave **3** in 77% yield over the two steps. The positions of acetal functionality in **2** and **3** were confirmed to be at C8 and C9 by acetylation of the product, yielding **2′** and **4**. In the ^1H NMR spectrum of **2′** and **4**, the signals for the C4 and C7 protons were shifted downfield; for example, comparing **2′** and **2**, the signal for H4 moved to 4.89 from 3.52 ppm, while that for H7 moved to 5.35 ppm from 3.48 ppm. Reductive cleavage of the benzylidene acetal in **4** upon reaction with borane dimethylamine complex and aluminum chloride afforded **5** in 49% yield. The same transformations used to convert **1** into **5** were used (Scheme 2) to synthesize acceptor **13** from the NTFA sialyl thioglycoside **6**,[3] via intermediates **9** and **10** in 65% overall yield.

In addition, **6** was also the precursor to the 1,5-lactamized acceptors **8** and **13** (Scheme 2). Reaction of **6** with sodium methoxide and then isopropylidenation of the resulting tetrol yielded an 71% yield of **7**, which was then converted to lactam **8** in 99% upon treatment with sodium methoxide at reflux for 4 days. Similarly, lactam formation from **9** afforded **11**, which was then acetylated in 94% overall yield. The benzylidene acetal in **11** was reductively opened upon treatment with borane dimethylamine complex and aluminum chloride[8] yielding **12** in 74% yield.

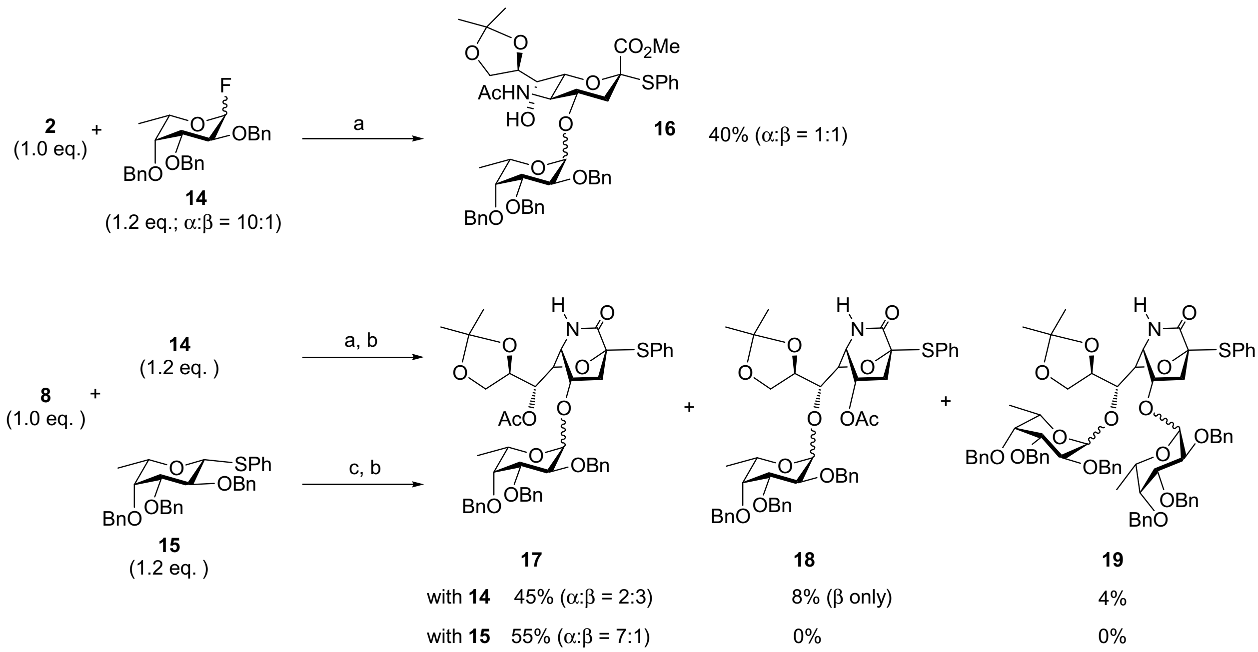


Scheme 2. Preparation of sialyl acceptors. Reagents and conditions: (a) (i) $NaOCH_3$, CH_3OH, rt, 24 h; (ii) 2,2-dimethoxypropane, CSA/CH_3CN, rt, 7 h, 71% (two steps); (b) $NaOCH_3$, CH_3OH, Drierite, reflux, 4 days, 99%; (c) (i) $NaOCH_3$, CH_3OH, rt, 29 h; (ii) $PhCH(OCH_3)_2$, CSA/DMF, 40 °C, 2 h, 88% (two steps); (d) (i) $NaOCH_3$, CH_3OH, Drierite, reflux, 5 days; (ii) Ac_2O, Py, DMAP, rt, 3 h; (iii) $NH_2NH_2{\cdot}AcOH$/THF, rt, 80 min, 94% (three steps); (e) $BH_3{\cdot}N(CH_3)_2$, $AlCl_3$/THF, 4 Å molecular sieves, 6 h, 0 °C→rt, 74%; (f) Ac_2O, pyridine, rt, quant.; (g) $BH_3{\cdot}N(CH_3)_2$, $AlCl_3$/THF, 4 Å molecular sieves, 3 h, 0 °C, 74%.

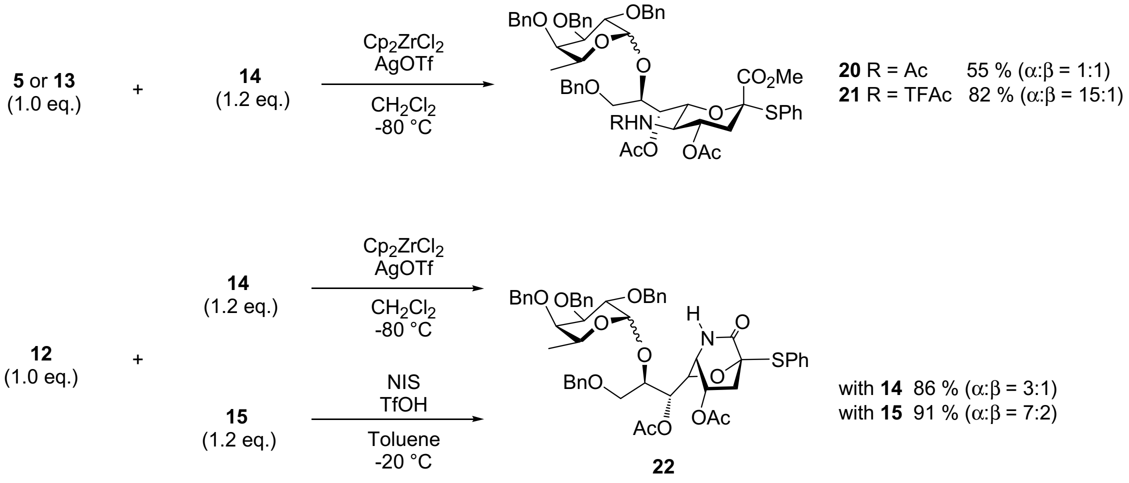
2.2. Glycosylation reactions

We initially glycosylated acceptors **2** and **8** (1.0 equiv each) with fucosyl donors **14**[9] and **15**[9] (1.2 equiv each) as shown in Scheme 3. For **2**, the fluoride donor **14** was selected as an orthogonal coupling partner.[10] Among various activation systems for fluoride donors, the use of the Suzuki method[11] (Cp_2HfCl_2/AgOTf) and 2,6-lutidine as an acid scavenger successfully activated fucosyl donor **14** in CH_2Cl_2 at −40 °C to rt without destroying the 8,9-*O*-isopropylidene moiety of **2**, thus producing the α-(1→4)-fucosyl sialoside in 40% yield as a α/β-mixture. However, the α- and β-isomers were inseparable by silica gel column chromatography. For the 1,5-lactamized acceptor **8**, thioglycoside **15** could serve as the donor due to the inactive phenylthio group on the bridge-head carbon of **8**.[4] The coupling reaction between **8** and **14** produced a complex mixture, which was acetylated to facilitate chromatographic separation. For this reaction disaccharide **17** was obtained in a yield of 45% as an α/β-mixture, together with the β-(2→7)-fucosyl disaccharide **18** (8%) and the double fucosylated product **19** (4%). The structure of product **19** was based on mass spectroscopic analysis. This compound showed a peak at $m/z = 1236.56$ in the MALDI MS spectrum, which corresponds to the $[M+Na]^+$ adduct (calculated for $C_{72}H_{79}NO_{14}S = 1236.51$). In contrast, donor **15** was regio- and stereoselectively glycosylated **8** upon activation with *N*-iodosuccinimide (NIS) and trifluoromethanesulfonic acid (TfOH)[12] to produce **17** in 48% yield, accompanied with slight amount of the β-isomer (7%).

The poor stereoselectivity observed in the reaction of the NAc-containing acceptor **2** and donor **14** was seen in the glycosylation of another NAc-containing acceptor, **5** (Scheme 4). In addition, the reaction of **5** and **14** provided an α/β mixture that was inseparable. Fortunately, a better result was obtained in the reaction of the NTFA-protected acceptor **13** with fucosyl donor **14**. This glycosylation furnished the α-Fuc*p*-(1→8)-Neu*p* sequence, with the α-anomer predominating, but chromatographic separation was again demanding. In contrast, fucosylation of the 1,5-lactamized-8-hydroxy acceptor **12** with donors **14** and **15** successfully delivered disaccharides. In both cases, although the stereoselectivity was moderate, anomer separation was readily achieved to afford pure α- and β-glycosides (Scheme 4). Overall, these results show that the 1,5-lactamized acceptors **8** and **12** are the best acceptors in α-(1→4)- and α-(1→8)-fucosylations, respectively. The stereochemistry of all glycosylations was determined from $J_{1,2}$ coupling constants in ^1H NMR spectra of the products (for the α-glycosides $J_{1,2} = 3.3–4.0$ Hz and for the β- glycosides: $J_{1,2} = 7.6–10.5$ Hz).



Scheme 3. Examination of α-(1→4)-fucosylation of sialic acid acceptors. Reagents and conditions: (a) Cp_2HfCl_2, AgOTf, 2,6-lutidine/CH_2Cl_2, 4 Å molecular sieves, −80 °C→− 10 °C; (b) Ac_2O, pyridine; (c) NIS, TfOH/CH_2Cl_2, 4 Å molecular sieves, −40 °C, 5 min.



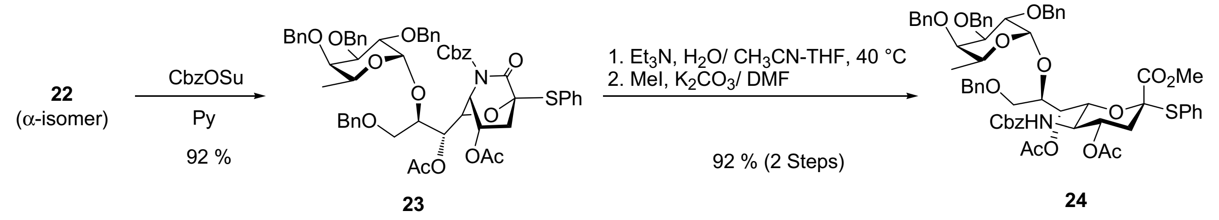
Scheme 4. Examination of α-(1→8)-fucosylation of sialic acid acceptors.

Finally, the protected α-Fuc*p*-(1→8)-Neu*p* derivative bearing 1,5-lactam ring (**22**) was transformed into an active disaccharide glycosyl donor by following the protocol developed[4] for the α-Neu*p*-(2→8)-Neu*p* structural motif (Scheme 5). Thus, the benzyloxycarbonyl (Cbz) group was installed onto the amide moiety yielding an 92% yield of **23**, which was then subjected to alkaline hydrolysis and methylation with methyl iodide, thereby providing disaccharide donor **24** in 92% yield over the two steps.

2.3. Conclusions

In conclusion, for the α-(1→4)- and α-(1→8)-fucosylation of sialic acid, 1,5-lactamized derivatives **8** and **12** serve as highly reactive glycosyl acceptors as was demonstrated in the case of α-(1→4)- and α-(1→8)-sialylations. The excellent results obtained from the coupling of NTFA-protected acceptor **13** support earlier observations made by the Boons' group,[13] which showed that the reactivity of the C8 hydroxyl group is increased when a trifluoroacetate group protects the C5 amino group.

To the best of our knowledge, this is the first report on the fucosylation of sialic acid. We note that in spite of the high reactivity of fucosyl donors as shown by many oligosaccharide syntheses,[9,14] the attachment of fucose to the C4 and C8 positions of sialic acid requires the use of optimized sialyl acceptors. These results suggest



Scheme 5. Transformation of **22** into disaccharide donor **24**.

that, with the exception of the primary C9 hydroxyl group, the other hydroxyl groups in sialic acid are inherently poorly reactive. In this context, the 1,5-lactamized acceptor should also be of use for other glycosyl modifications of sialic acid residues found in nature, including those linked to galactose[1b] and galactosamine[15] residues. In preliminary studies, we have succeeded in the synthesis of the tetrasaccharide portion of HPG-1 by use of donor **24**. This result of this study will be reported in the near future.

3. Experimental

3.1. General procedures

^{1}H and ^{13}C NMR spectra were recorded on Varian Unity INOVA 400, 500 and Jeol ECA 500 instruments. Chemical shifts are expressed in parts per million (δ) relative to the signal of either $CHCl_3$ or $(CH_3)_4Si$, adjusted to 7.26 or 0.00 ppm, respectively. MALDI-TOFMS spectra were recorded in positive ion mode on a Brucker Autoflex with the use of α-cyano-4-hydroxy-cinnamic acid (CHCA) as a matrix. Molecular sieves were purchased from Wako Chemicals Inc. and dried at 300 °C for 2 h in a muffle furnace prior to use. Drierite was powdered and dried at 300 °C for 6 h in a muffle furnace prior to use. Reaction solvents were dried over molecular sieves and used without purification. TLC analysis was performed on Merck TLC (Silica Gel $60F_{254}$ on glass plates). Silica gel (80 mesh and 300 mesh) manufactured by Fuji Silysia Co. was used for flash column chromatography. The quantity of silica gel was usually estimated as 100- to 150-fold weight of sample to be charged. Solvent systems in chromatography are specified in v/v. Evaporation and condensations were carried out in vacuo.

3.2. Methyl (phenyl 5-acetamido-3,5-dideoxy-8,9-*O*-isopropylidene-2-thio-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (2)

To a solution of methyl (phenyl 4,7,8,9-tetra-*O*-acetyl-5-acetamido-3,5-dideoxy-2-thio-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (**1**, 5.00 g, 8.57 mmol) in CH_3OH (170 mL), a 28% solution of sodium methoxide in CH_3OH (166 mg, 0.86 mmol) was added under argon. The mixture was stirred for 27 h at rt, with monitoring of the reaction by TLC (5:1, $CHCl_3/CH_3OH$). The reaction mixture was neutralized with Dowex HCR-W2-H(H^+), filtered, and concentrated. The resulting residue was dried in vacuo, dissolved in CH_3CN (171 mL) and warmed to 40 °C. To the solution, 2,2-dimethoxypropane (1.58 mL, 12.9 mmol) and ±10-camphorsulfonic acid (99.5 mg, 42.8 μmol) were added. The mixture was stirred for 1.5 h at ambient temperature until the starting material was disappeared by TLC (5:1, $CHCl_3/CH_3OH$). The reaction mixture was alkalized to pH $\sim$ 9 with Et_3N and co-evaporated with toluene. The residue was purified by column chromatography on silica gel (25:1, $CHCl_3/CH_3OH$) to give **2** (2.35 g, 60%): $[\alpha]_D$ −0.05 (*c* 0.1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.42–7.27 (m, 5H, 2Ph), 6.27 (d, 1H, NH), 4.19 (m, 1H, H-8), 4.14 (dd, 1H, $J_{8,9} = 6.2$ Hz, $J_{gem} = 8.4$ Hz, H-9), 3.95 (dd, 1H, $J_{8,9'} = 7.0$ Hz, H-9′), 3.85 (q, 1H, H-5), 3.66 (dt, 1H, $J_{3ax,4} = 11.0$ Hz, $J_{3eq,4} = 4.8$ Hz, H-4), 3.50 (s, 3H, Me), 3.47 (m, 1H, H-7), 3.25 (d, 1H, H-6), 2.91 (dd, 1H, $J_{gem} = 12.8$ Hz, H-3eq), 2.01 (s, 3H, NAc), 1.89 (t, 1H, H-3ax), 1.34, 1.24 (2s, 6H, 2Me); ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.2, 169.4, 136.7, 129.9, 129.1, 128.6, 108.6, 87.3, 76.7, 76.6, 74.9, 74.9, 70.3, 70.2, 68.3, 68.1, 67.2, 52.6, 52.5, 52.2, 40.8, 26.7, 25.3, 23.1, 23.1; MALDI-TOFMS *m*/*z* calcd for $C_{21}H_{29}NO_8S$ $[M+Na]^+$: 478.15. Found 478.02.

3.3. Methyl (phenyl 5-acetamido-4,7-di-*O*-acetyl-3,5-dideoxy-8,9-*O*-isopropylidene-2-thio-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (2′)

$[\alpha]_D$ +1.3 (*c* 0.1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.70–7.29 (m, 5H, Ph), 5.40 (d, 1H, NH), 5.29 (dd, 1H, $J_{6,7} = 4.8$ Hz, $J_{7,8} = 1.8$ Hz, H-7), 4.92 (td, 1H, $J_{3ax,4} = 11.0$ Hz, $J_{3eq,4} = 4.8$ Hz, H-4), 4.24 (m, 1H, $J_{8,9} = 6.6$ Hz, H-8), 3.97 (m, 2H, H-9 and H-5), 3.67 (dd, 1H, $J_{5,6} = 1.4$ Hz, H-6), 3.62 (s, 3H, Me), 2.81 (dd, 1H, $J_{gem} = 12.8$ Hz, H-3eq), 2.16–1.86 (m, 10H, H-3 ax, 3Ac), 1.31, 1.29 (2s, 6H, 2Me); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.9, 170.3, 170.2, 168.5, 136.8, 130.2, 128.8, 128.7, 108.5, 87.5, 75.7, 75.3, 69.5, 69.0, 66.0, 52.7, 49.5, 37.8, 26.4, 25.4, 23.1, 20.9, 20.8; MALDI-TOFMS *m*/*z* calcd for $C_{21}H_{29}NO_8S$ $[M+Na]^+$: 539.18. Found 539.18.

3.4. Methyl (phenyl 5-acetamido-8,9-*O*-benzylidene-3,5-dideoxy-2-thio-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (3)

To a solution of compound **1** (3.85 g, 6.6 mmol) in CH_3OH (132 mL), a 28% solution of sodium methoxide in CH_3OH (128 mg, 0.66 mmol) was added under an argon atmosphere. The mixture was stirred for 27 h at rt with monitoring of the reaction by TLC (5:1, $CHCl_3/CH_3OH$). The reaction mixture was neutralized with Dowex HCR-W2-H(H^+), filtered, and concentrated. The resultant residue was dried in vacuo, dissolved in DMF (73 mL) and warmed up 40 °C. To the solution, benzaldehydedimethylacetal (1.98 mL, 13.2 mmol) and ±10-camphorsulfonic acid (154 mg, 0.66 mmol) were added and stirring was continued for 18 h at 40 °C until the starting material was disappeared by TLC. The reaction mixture was alkalized to pH ~ 9 with Et_3N and concentrated. The resultant syrup was dissolved in $CHCl_3$, and washed with satd aq $NaHCO_3$, brine, and dried over Na_2SO_4. After concentration, the residue was purified by column chromatography on silica gel (20:1, $CHCl_3/CH_3OH$) to give **3** (2.31 g, 77%); ^1H NMR (400 MHz, $CDCl_3$): δ 7.51–7.20 (m, 10H, 2Ph), 4.21 (m, 1H, $J_{7,8} = 7.0$ Hz, $J_{8,9} = 7.0$ Hz, $J_{8,9'} = 5.1$ Hz, H-8), 4.12 (dd, 1H, $J_{gem} = 8.4$ Hz, H-9′), 3.91 (m, 1H, H-9), 3.71 (m, 1H, H-5), 3.56–3.37 (m, 2H, H-4 and H-7), 3.32–3.30 (m, 4H, H-6 and Me), 2.71 (dd, 1H, $J_{gem} = 12.5$ Hz, $J_{3eq,4} = 4.8$ Hz, H-3eq), 1.85 (s, 3H, 3Ac), 1.69 (t, 1H, $J_{3ax,4} = 11.7$ Hz, H-3ax); ^{13}C NMR (100 MHz, CD_3OD): δ 173.4, 169.1, 137.8, 136.5, 129.6, 129.4, 129.0, 128.4, 127.9, 126.5, 103.8, 87.3, 76.2, 75.9, 69.7, 67.6, 67.5, 52.1, 51.3, 48.3, 48.1, 47.9, 47.7, 47.5, 47.3, 47.1, 40.6, 21.4; MALDI-TOFMS *m*/*z* calcd for $C_{25}H_{29}NO_8S$ $[M+Na]^+$: 526.56. Found 526.25.

3.5. Methyl (phenyl 5-acetamido-4,7-di-*O*-acetyl-8,9-*O*-benzylidene-3,5-dideoxy-2-thio-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (4)

Compound **3** (2.31 g, 5.1 mmol) was acetylated with Ac_2O and pyridine under conventional conditions to give **4** (2.99 g, quant.); ^1H NMR (400 MHz, $CDCl_3$): δ 7.58–7.27 (m, 10H, 2Ph), 5.35 (dd, 1H, $J_{7,8} = 4.8$ Hz, $J_{6,7} = 2.2$ Hz, H-7), 5.21 (d, 1H, NH), 4.89 (m, 1H, H-4), 4.42 (m, 1H, H-8), 4.17 (dd, 1H, $J_{gem} = 8.4$ Hz, $J_{8,9} = 5.5$ Hz, H-9), 3.99–3.91 (m, 2H, H-5, H-9′), 3.71 (dd, 1H, H-6), 3.52 (s, 3H, Me), 2.80 (dd, 1H, $J_{gem} = 12.8$ Hz, $J_{3eq,4} = 4.8$ Hz, H-3eq), 2.08–1.87 (m, 10H, H-3ax and 3Ac); ^{13}C NMR (100 MHz, CD_3OD): δ 170.7, 170.3, 170.2, 168.5, 137.4, 136.8, 130.2, 129.2, 128.5, 128.3, 126.3, 103.3, 94.3, 87.4, 76.0, 75.6, 70.0, 68.8, 66.8, 52.7, 49.2, 37.8, 23.1, 20.8, 20.8; MALDI-TOFMS *m*/*z* calcd for $C_{29}H_{33}NO_{10}S$ $[M+Na]^+$: 610.63. Found 610.48.

3.6. Methyl (phenyl 5-acetamido-4,7-di-*O*-acetyl-9-*O*-benzyl-3,5-dideoxy-2-thio-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (5)

To a mixture of compound **4** (251 mg, 0.427 mmol) and 4 Å molecular sieves (1.5 g) in THF (5.0 mL), $BH_3{\cdot}NMe_3$ (193 mg, 2.65 mmol) and $AlCl_3$ (342 mg, 2.56 mmol) were added at 0 °C. The reaction mixture was stirred for 1 h at 0 °C and for another 1 h at rt (TLC; 10:1, $CHCl_3/CH_3OH$). $AlCl_3$ (228 mg, 1.70 mmol) was added to the reaction mixture, and stirred for 2.5 h at rt and for 0.5 h at 45 °C. The reaction mixture was filtered through Celite and the combined filtrate and washings were extracted with EtOAc. The organic layer was washed with satd aq $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated. The residue was purified by column chromatography on silica gel (50:1, $CHCl_3/CH_3OH$) to give **12** (126 mg, 49%); $[\alpha]_D$ +5.8 (*c* 1.1, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 7.54–7.26 (m, 10H, 2Ph), 5.34 (d, 1H, NH), 5.10 (dd, 1H, $J_{6,7} = 2.2$ Hz, H-7), 4.79 (td, 1H, $J_{3eq,4} = 4.8$ Hz, $J_{3ax,4} = 11.4$ Hz, $J_{4,5} = 10.6$ Hz, H-4), 4.59–4.48 (m, 2H, $PhCH_2$), 4.14 (q, 1H, $J_{5,6} = 10.6$ Hz, H-5), 4.06 (m, 1H, H-8), 3.74 (dd, 1H, H-6), 3.69 (s, 3H, Me), 3.50 (dd, 1H, $J_{gem} = 9.5$ Hz, H-9), 3.42 (dd, 1H, H-9′), 2.86 (dd, 1H, $J_{gem} = 12.8$ Hz, H-3eq), 2.10–2.03 (m, 7H, H-3ax and 2Ac); ^{13}C NMR (100 MHz, CD_3OD): δ 170.8, 170.3, 170.2, 169.3, 138.1, 136.7, 130.5, 128.9, 128.2, 128.0, 127.9, 127.5, 86.5, 75.1, 73.4, 70.8, 70.1, 69.3, 69.3, 53.3, 48.6, 37.3, 30.0, 23.1, 21.0, 20.8; MALDI-TOFMS *m*/*z* calcd for $C_{29}H_{35}NO_{10}S$ $[M+Na]^+$: 612.64. Found 612.26.

3.7. Methyl (phenyl 3,5-dideoxy-8,9-*O*-isopropylidene-2-thio-5-trifluoroacetamido-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (7)

To a solution of compound **6** (5.34 g, 8.4 mmol) in CH_3OH (100 mL), a 28% solution of sodium methoxide in CH_3OH (162 mg, 0.84 mmol) was added under an argon atmosphere. The mixture was stirred for 24 h at rt, with monitoring of the reaction by TLC (10:1, $CHCl_3/CH_3OH$). The reaction mixture was neutralized with Dowex HCR-W2-H(H^+), filtered, and concentrated. The resultant residue was dried in vacuo, dissolved in DMF (157 mL) and warmed up 40 °C. To the solution, 2,2-dimethoxypropane (1.45 mL, 11.8 mmol) and ±10-camphorsulfonic acid (91 mg, 0.39 mmol) were added and stirring was continued for 7 h at rt until the starting material disappeared by TLC. The reaction mixture was alkalized to pH ~ 9 with Et_3N and concentrated. The residue was purified by column chromatography on silica gel (2:1, *n*-hexane/EtOAc) to give **7** (2.84 g, 71%); $[\alpha]_D$ −1.0 (*c* 0.2, $CHCl_3$); ^1H NMR (400 MHz, CD_3OD): δ 7.34–7.44 (m, 5H, Ph), 4.62 (d, 1H, NH), 4.14 (m, 1H, H-8), 3.95 (m, 3H, H-5, H-6, and H-7), 3.67 (m, 1H, H-4), 3.58 (dd, 1H, $J_{8,9} = 1.1$ Hz, $J_{gem} = 10.5$ Hz, H-9), 3.47 (s, 3H, Me), 3.44 (m, 1H, H-9′), 2.81 (dd, 1H, $J_{3eq,4} = 4.8$ Hz, $J_{gem} = 12.8$ Hz, H-3eq), 1.80 (t, 1H, $J_{3ax,4} = 11.4$ Hz, H-3ax), 1.30, 1.22 (2s, 6H, 2Me); ^{13}C NMR (100 MHz, CD_3OD): δ 170.2, 137.9, 130.9, 130.5, 129.6, 109.8, 88.6, 76.8, 76.3, 70.7, 68.6, 67.7, 53.5, 52.6, 41.8, 27.0, 25.7, 14.5; MALDI-TOFMS *m*/*z* calcd for $C_{21}H_{29}F_3NO_8S$ $[M+H]^+$: 477.18. Found 478.34.

3.8. Methyl (phenyl 4,7-di-*O*-acetyl-3,5-dideoxy-8,9-*O*-isopropylidene-2-thio-5-trifluoroacetamido-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (7′)

$[\alpha]_D$ +1.3 (*c* 0.1, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 7.27–7.57 (m, 5H, Ph), 6.47 (d, 1H, NH), 5.20 (dd, 1H, $J_{6,7} = 5.2$ Hz, $J_{7,8} = 1.5$ Hz, H-7), 5.09 (td, 1H, $J_{3eq,4} = 5.1$ Hz, $J_{3ax,4} = 11.4$ Hz, $J_{4,5} = 11.4$ Hz, H-4), 4.28 (dd, 1H, $J_{5,6} = 6.6$ Hz, H-6), 3.94 (m, 3H, H-5, H-8, and H-9), 3.82 (dd, 1H, $J_{8,9'} = 1.5$ Hz, $J_{gem} = 11.1$ Hz, H-9′), 3.62 (s, 3H, Me), 2.87 (dd, 1H, $J_{gem} = 12.8$ Hz, H-3eq), 2.16 and 2.02 (2s, 6H, 2Ac), 1.97 (dd, 1H, H-3ax), 1.32, 1.29 (2s, 6H, 2Me); ^{13}C NMR (100 MHz, $CDCl_3$): 170.9, 170.1, 168.2, 157.7, 157.3, 136.8, 128.8, 128.4, 108.7, 87.5, 74.8, 74.7, 69.1, 68.8, 66.0, 52.8, 50.3, 37.6, 26.3, 25.4, 20.7, 20.6.

3.9. Phenyl 5-amino-3,5-dideoxy-8,9-*O*-isopropylidene-2-thio-D-*glycero*-α-D-*galacto*-2-nonulopyranoside-1,5-lactam (8)

To a solution of compound **7** (2.39 g, 5.0 mmol) in CH_3OH (615 mL), Drierite (6.0 g) and a 28% solution of sodium methoxide were added in CH_3OH (2.8 g, 14.8 mmol) under an argon atmosphere. The suspension was stirred for 4 days at reflux while the reaction was monitored by TLC (5:1, $CHCl_3/CH_3OH$). The reaction mixture was neutralized with Dowex HCR-W2-H(H^+) and filtered through Celite. The combined filtrate and washings were concentrated and the resulting residue was purified by column chromatography on silica gel (15:1, $CHCl_3/CH_3OH$) to give **8** (1.89 g, 99%); $[\alpha]_D$ −6.8 (*c* 0.2, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$): δ 7.62–7.28 (m, 5H, Ph), 4.28 (s, 1H, H-6), 4.09 (m, 2H, H-4 and H-8), 3.95 (dd, 1H, $J_{6,7} = 12.8$ Hz, $J_{7,8} = 6.2$ Hz, H-7), 3.78 (near d, 1H, $J_{5,6} = 5.9$ Hz, H-5), 3.67 (m, 2H, H-9 and H-9′), 2.87 (t, 1H, $J_{3eq,4} = 11.0$ Hz, $J_{gem} = 13.9$ Hz, H-3eq), 1.83 (dd, 1H, $J_{3ax,4} = 3.7$ Hz, H-3ax), 1.36, 1.26 (2s, 6H, 2Me); ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.6, 170.5, 136.4, 129.3, 128.8, 109.7, 85.7, 78.3, 76.7, 76.0, 70.4, 67.2, 66.9, 53.7, 53.6, 41.0, 29.6, 26.5, 25.2; MALDI-TOFMS *m*/*z* calcd for $C_{18}H_{23}NO_6S$ $[M+H]^+$: 382.12. Found 382.16.

3.10. Phenyl 5-acetamido-4,7-di-*O*-acetyl-3,5-dideoxy-8,9-*O*-isopropylidene-2-thio-D-*glycero*-α-D-*galacto*-2-nonulopyranoside-1,5-lactam (8′)

$[\alpha]_D$ +4.7 (*c* 0.1, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 7.58–7.21 (m, 5H, Ph), 5.62 (dd, 1H, $J_{7,8} = 5.9$ Hz, $J_{6,7} = 8.1$ Hz, H-7), 5.31 (dd, 1H, $J_{4,5} = 3.3$ Hz, $J_{5,6} = 1.8$ Hz, H-5), 4.92 (m, 1H, H-4), 4.12 (m, 1H, H-8), 4.07 (dd, 1H, H-6), 4.01 (dd, 1H, $J_{8,9} = 6.2$ Hz, $J_{gem} = 8.1$ Hz, H-9), 3.80 (dd, 1H, $J_{8,9'} = 7.3$ Hz, H-9′), 2.50 (m, 1H, H-3eq), 2.11 (dd, 1H, $J_{3ax,4} = 2.2$ Hz, H-3ax), 2.07 and 2.06 (2s, 6H, 2Ac), 1.38 and 1.34 (2s, 6H, 2Me); ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.5, 169.4, 169.0, 167.2, 136.6, 129.5, 128.8, 128.3, 109.8, 86.8, 74.7, 70.9, 67.4, 65.7, 48.6, 37.9, 29.7, 26.5, 26.2, 25.2, 21.0, 20.8; MALDI-TOFMS *m*/*z* calcd for $C_{24}H_{29}NO_9S$ $[M+Na]^+$: 530.14. Found 530.44.

3.11. Methyl (phenyl 8,9-*O*-benzylidene-3,5-dideoxy-2-thio-5-trifluoroacetamido-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (9)

To a solution of compound **6** (300 mg, 0.47 mmol) in CH_3OH (9.4 mL), a 28% solution of sodium methoxide in CH_3OH (8.9 mg, 47 μmol) was added under an argon atmosphere. The mixture was stirred for 29 h at rt, while the reaction was monitored by TLC (10:1, $CHCl_3/CH_3OH$). The reaction mixture was neutralized with Dowex HCR-W2-H(H^+), filtered, and concentrated. The resultant residue was dried in vacuo, dissolved in DMF (5.2 mL), and warmed up 40 °C. To the solution, benzaldehydedimethylacetal (141 μmol) and ±10-camphorsulfonic acid (11 mg, 47 μmol) were added and stirring was continued for 2 h at 40 °C until the starting material disappeared by TLC. The reaction mixture was alkalized to pH ∼ 9 with Et_3N and co-evaporated with toluene. The residue was purified by column chromatography on silica gel (1:2, *n*-hexane/EtOAc) to give **11** (230 mg, 88%); ^1H NMR (400 MHz, $CDCl_3$): δ 7.55–7.22 (m, 10H, 2Ph), 5.69 (s, 1H, PhC*H*), 3.68 (s, 1H, H-4), 2.88 (m, 1H, H-3ax), 1.91 (m, 1H, H-3eq); ^{13}C NMR (100 MHz, $CDCl_3$): δ 136.8, 134.5, 129.7, 129.0, 128.8, 128.3, 126.2; MALDI-TOFMS *m*/*z* calcd for $C_{25}H_{26}F_3NO_8S$ $[M+Na]^+$: 580.12. Found 580.35.

3.12. Methyl (phenyl 4,7-di-*O*-acetyl-8,9-*O*-benzylidene-3,5-dideoxy-2-thio-5-trifluoroacetamido-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (10)

Compound **9** (230 mg, 0.41 mmol) was acetylated with Ac_2O and pyridine by conventional means to give **10** (262 mg, quant.): ^1H NMR (400 MHz, $CDCl_3$): δ 7.59–7.34 (m, 10H, 2Ph), 6.24 (d, 1H, NH), 5.84 (s, 1H), 5.22

(dd, 1H, $J_{7,8} = 6.6$ Hz, H-7), 5.05 (m, 1H, H-4), 4.46 (m, 1H, H-8), 4.16 (dd, 1H, H-9), 4.00–3.85 (m, 3H, H-9′, H-5, and H-6), 3.43 (s, 3H, Me), 2.90 (dd, 1H, H-3eq), 2.22–1.96 (m, 7H, H-3ax and 2Ac); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.8, 170.6, 170.3, 170.0, 167.6, 136.4, 130.0, 128.9, 87.5, 73.8, 70.1, 69.0, 67.5, 61.8, 52.8, 50.1, 38.0, 20.9, 20.7, 20.6, 20.5; MALDI-TOFMS *m/z* calcd for $C_{29}H_{30}F_3NO_{10}S$ $[M+Na]^+$: 664.14. Found 664.24.

3.13. Phenyl 4,7-di-*O*-acetyl-5-amino-9-*O*-benzylidene-3,5-dideoxy-2-thio-D-*glycero*-α-D-*galacto*-2-nonulopyranoside-1,5-lactam (11)

To a solution of compound **9** (1.59 g, 2.86 mmol) in CH_3OH (300 mL), Drierite (3.4 g) and a 28% solution of sodium methoxide were added in CH_3OH (1.37 g, 7.1 mmol) under an argon atmosphere. The suspension was stirred for 5 days at reflux as the reaction was monitored by TLC (10:1, $CHCl_3/CH_3OH$). The reaction mixture was filtered through Celite, the combined filtrate and washings were concentrated and the residue was purified by column chromatography on silica gel (80:1→70:1→60:1→40:1→5:1, $CHCl_3/CH_3OH$) to give the lactam derivative. To the solution of lactam in pyridine (2.1 mL), Ac_2O (1.62 mL, 17.2 mmol) and 4-dimethylaminopyridine (35.0 mg, 0.286 mmol) were added, and the mixture was stirred for 3 h. The reaction was monitored by TLC (1:2, *n*-hexane/EtOAc). The reaction mixture was co-evaporated with toluene and diluted with EtOAc. The organic phase was washed with 2 M HCl, water, satd aq Na_2CO_3, and brine, dried (Na_2SO_4), and concentrated. The resulting residue was dissolved in THF (57 mL) followed by the addition of hydrazine acetate (264 mg, 2.86 mmol). The mixture was stirred for 80 min at rt, diluted with EtOAc, washed with 2 M HCl, satd aq $NaHCO_3$, and brine, dried (Na_2SO_4), and concentrated. The residue was purified by chromatography on silica gel (1:1, *n*-hexane/EtOAc) to give **11** (1.38 g, 94%): ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.55–7.27 (m, 10H, 2Ph), 5.76 (s, 1H, PhC*H*), 5.72 (m, 1H, H-7), 5.04 (m, 1H, H-4), 3.95 (dd, 1H, $J_{gem} = 8.0$ Hz, $J_{8,9} = 6.6$ Hz, H-9), 2.52 (dd, $J_{gem} = 14.2$ Hz, $J_{3eq,4} = 10.6$ Hz, H-3eq), 2.14 and 2.05 (2s, 6H, 2Ac); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.5, 170.4, 169.8, 169.7, 169.3, 169.3, 137.2, 136.4, 136.3, 129.2, 129.2, 128.7, 128.3, 128.2, 128.1, 126.0, 126.0, 104.2, 104.0, 85.7, 79.4, 79.0, 75.6, 74.5, 71.2, 71.1, 68.8, 68.4, 67.8, 66.9, 49.2, 49.1, 37.8, 37.8, 21.0, 20.9, 20.9, 20.5.

3.14. Phenyl 5-amino-4,7-di-*O*-acetyl-9-*O*-benzyl-3,5-dideoxy-2-thio-D-*glycero*-α-D-*galacto*-2-nonulopyranoside-1,5-lactam (12)

To a mixture of compound **11** (82.0 mg, 0.16 mmol) and 4 Å molecular sieves (500 mg) in THF (1.9 mL), $BH_3{\cdot}NMe_3$ (72 mg, 0.99 mmol) and $AlCl_3$ (128 mg, 0.96 mmol) were added at 0 °C, and then the mixture was stirred for 2 h at 0 °C and for another 4 h at rt (TLC; 10:1, $CHCl_3/CH_3OH$). The reaction mixture was filtered through Celite, the combined filtrate and washings were extracted with Et_2O. The organic layer was washed with satd aq $NaHCO_3$ and brine before being dried (Na_2SO_4) and concentrated. The residue was purified with column chromatography on silica gel (60:1, $CHCl_3/CH_3OH$) to give **12** (61 mg, 74%): $[\alpha]_D$ 12.0 (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.55–7.25 (m, 10H, 2Ph), 5.51 (dd, 1H, H-7), 5.00 (m, 1H, H-4), 4.49 (m, 2H, PhC*H*$_2$), 4.26 (m, 2H, H-5 and H-6), 3.80 (m, 1H, H-8), 3.51 (dd, 1H, $J_{gem} = 9.5$ Hz, H-9), 3.36 (dd, 1H, H-9′), 2.46 (dd, 1H, $J_{gem} = 14.4$ Hz, H-3ax), 2.12 (s, 3H, NAc), 2.05 (dd, 1H, H-3eq), 1.89 (s, 3H, Ac); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.1, 170.1, 169.9, 137.2, 136.2, 129.0, 128.6, 128.5, 128.4, 128.0, 85.6, 79.4, 77.2, 73.5, 72.1, 70.3, 69.8, 68.6, 49.2, 37.5, 21.0, 20.9; MALDI-TOFMS *m/z* calcd for $C_{26}H_{27}NO_8S$ $[M+Na]^+$: 538.16. Found 538.15.

3.15. Methyl (phenyl 4,7-di-*O*-acetyl-9-*O*-benzyl-3,5-dideoxyl-2-thio-5-trifluoroacetamido-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (13)

To a mixture of compound **10** (2.27 g, 3.5 mmol) and 4 Å molecular sieves (13.6 g) in THF (41 mL), $BH_3{\cdot}NMe_3$ (1.58 g, 21.7 mmol) and $AlCl_3$ (2.8 g, 0.42 mol) were added at 0 °C, and then the mixture was stirred for 3 h (TLC; 10:1, $CHCl_3/CH_3OH$). The reaction mixture was filtered through Celite and the combined filtrate and washings were extracted with EtOAc. The organic layer was washed with satd aq $NaHCO_3$ and brine, dried (Na_2SO_4), and concentrated. The residue was purified by column chromatography on silica gel (100:1, $CHCl_3$/MeOH) to give **12** (1.70 g, 74%): $[\alpha]_D$ +40.6 (*c* 1.1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.52–7.25 (m, 10H, 2Ph), 6.69 (d, 1H, NH), 5.08 (dd, 1H, $J_{6,7} = 2.2$ Hz, $J_{7,8} = 8.8$ Hz, H-7), 4.90 (m, 1H, H-4), 4.56–4.46 (2d, 2H, PhC*H*$_2$), 4.13–4.02 (m, 2H, H-5 and H-8), 3.88 (dd, 1H, H-6), 3.67 (s, 3H, Me), 3.46 (dd, 1H, $J_{8,9} = 5.9$ Hz, $J_{gem} = 10.6$ Hz, H-9), 3.36 (dd, 1H, $J_{8,9'} = 3.3$ Hz, H-9′), 2.92 (dd, 1H, $J_{3eq,4} = 4.8$ Hz, $J_{gem} = 12.8$ Hz, H-3eq), 2.09–2.00 (m, 7H, H-3ax and 2Ac); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.9, 170.3, 170.2, 169.2, 138.2, 137.0, 130.8, 129.2, 128.5, 128.2, 128.1, 127.8, 86.7, 74.6, 73.7, 70.9, 69.6, 69.3, 53.7, 49.9, 37.5, 21.0, 20.7; MALDI-TOFMS *m/z* calcd for $C_{29}H_{32}F_3NO_{10}S$ $[M+Na]^+$: 666.16. Found 666.17.

3.16. Phenyl [5-acetamido-3,5-dideoxy-8,9-*O*-isopropylidene-2-thio-4-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-D-*glycero*-D-*galacto*-2-nonulopyranosid]onate (16)

To a solution of **2** (110 mg, 0.22 mmol) and **14** (115 mg, 0.26 mmol) in CH_2Cl_2 (4.8 mL), 4 Å molecular sieves

(150 mg) was added. The suspension was stirred for 1 h and cooled to −20 °C. To the mixture, 2,6-lutidine (30 μL, 0.26 mmol), Cp_2HfCl_2 (250 mg, 0.65 mmol), and AgOTf (338 mg, 1.30 mmol) were added and stirring was continued for 2 h until TLC analysis (15:1, $CHCl_3/CH_3OH$) indicated the completion of the reaction. The reaction mixture was alkalized to pH ∼ 8 with satd aq $NaHCO_3$ at 0 °C and filtered through Celite. The combined filtrate and washings were extracted with $CHCl_3$ and the organic layer was washed with water, dried (Na_2SO_4), and concentrated. The residue was purified by column chromatography on silica gel (75:1, $CHCl_3/CH_3OH$) to give disaccharide **16** (76 mg, 40%) as an α/β-mixture (α:β = 1:1).

3.17. Phenyl 7-*O*-acetyl-5-amino-3,5-dideoxy-8,9-*O*-isopropylidene-2-thio-4-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-D-*glycero*-α-D-*galacto*-2-nonulopyranoside-1,5-lactam (17)

With fucosyl fluoride donor **14**: To a solution of compound **8** (110 mg, 0.29 mmol) and compound **14** (152 mg, 0.35 mmol) in CH_2Cl_2 (5.3 mL) was added 4 Å molecular sieves (150 mg). The suspension was stirred for 1 h and cooled to −40 °C. To the mixture were added 2,6-lutidine (40 μL, 0.35 mmol), Cp_2HfCl_2 (148 mg, 0.42 mmol), and AgOTf (100 mg, 0.42 mmol) and stirring was continued for 3 h. After the addition of 2,6-lutidine (40 μL, 0.35 mmol), Cp_2HfCl_2 (148 mg, 0.42 mmol), and AgOTf (100 mg, 0.42 mmol), stirring was continued for 3 h at −10 °C until TLC analysis (15:1, $CHCl_3/CH_3OH$) indicated the completion of the reaction. The reaction mixture was alkalized to pH ∼ 8 with satd aq $NaHCO_3$ at 0 °C and filtered through Celite. The combined filtrate and washings were extracted with $CHCl_3$, and the organic layer was washed with water, dried (Na_2SO_4), and concentrated. The residue was purified by column chromatography on silica gel (5:2, toluene/EtOAc) to give disaccharide as a α/β-mixture (α:β = 2:3), which was acetylated with Ac_2O and pyridine to afford **17α** (48 mg, 18%) and the corresponding β-isomer **17β** (71 mg, 27%).

With phenylthioglycoside donor **15**: To a solution of **8** (110 mg, 0.29 mmol) and **15** (184 mg, 0.35 mmol) in CH_2Cl_2 (5.3 mL), 4 Å molecular sieves (150 mg) was added. The suspension was stirred for 1 h and cooled to −40 °C. To the mixture, NIS (95 mg, 0.42 mmol) and TfOH (3.7 μL, 42 μmol) were added and stirring was continued for 5 min. The reaction mixture was alkalized to pH ∼ 8 with satd aq $NaHCO_3$ at 0 °C and filtered through Celite. The combined filtrate and washings were extracted with EtOAc, and the organic layer was washed with satd aq $Na_2S_2O_3$ and brine, dried (Na_2SO_4), and concentrated. The residue was purified by column chromatography on silica gel (75:1, $CHCl_3/CH_3OH$) to give the disaccharide as an α/β-mixture (α:β = 7:1), which was acetylated with Ac_2O and pyridine to afford **17α** (116 mg, 48%) and **17β** (16 mg, 7%); **17α**: $[\alpha]_D$ −13.3 (*c* 1.0, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$): δ 7.57–7.26 (m, 20H, 4Ph), 5.99 (dd, 1H, $J_{6,7} = 7.4$ Hz, $J_{7,8} = 9.7$ Hz, H-7_{Neu}), 5.32 (t, 1H, $J_{4,5} = 2.3$ Hz, $J_{5,6} = 2.3$ Hz, H-5_{Neu}), 4.85 (s, 1H, $J_{1,2} = 4.0$ Hz, H-1_{Fuc}), 4.81–4.62 (m, 7H, H-6_{Neu}, 3PhCH_2), 4.64 (dd, 1H, $J_{2,3} = 7.4$ Hz, H-2_{Fuc}), 4.28 (dd, 1H, $J_{8,9} = 7.4$ Hz, H-9_{Neu}), 3.84–3.89 (m, 3H, H-4_{Neu}, H-8_{Neu}, and H-3_{Fuc}), 3.41 (dd, 1H, $J_{4,5} = 7.4$ Hz, $J_{5,6} = 6.3$ Hz, H-5_{Fuc}), 2.13 (s, 6H, Ac), 2.45 (dd, 1H, $J_{gem} = 14.9$ Hz, $J_{3eq,4} = 10.9$ Hz, H-$3eq_{Neu}$), 2.28 (dd, 1H, $J_{3ax,4} = 5.7$ Hz, H-$3ax_{Neu}$), 1.17 (d, 1H, H-6_{Fuc}), 1.28 and 1.16 (2s, 6H, 2Me); ^{13}C NMR (125 MHz, $CDCl_3$): δ 169.3, 138.5, 138.5, 135.8, 129.0, 128.8, 128.7, 128.4, 128.4, 128.4, 128.2, 128.1, 127.6, 127.5, 127.5, 127.4, 108.9, 104.6, 85.9, 82.1, 78.9, 78.1, 76.3, 75.7, 75.5, 74.7, 73.6, 73.2, 71.6, 70.9, 64.0, 51.5, 38.5, 29.7, 26.1, 24.9, 21.1, 16.9; MALDI-TOFMS *m*/*z* calcd for $C_{47}H_{53}NO_{11}S$ $[M+Na]^+$: 862.32. Found 862.34. **17β**: ^1H NMR (500 MHz, $CDCl_3$): δ 7.53–7.26 (m, 20H, 4Ph), 5.97 (dd, 1H, $J_{6,7} = 10.3$ Hz, H-7_{Neu}), 5.15 (m, 1H, H-5_{Neu}), 4.89 (dd, 1H, $J_{1,2} = 11.5$ Hz, H-1_{Fuc}), 4.54 (m, 8H, H-8_{Neu}, H-2_{Fuc} and 3PhCH_2), 3.91 (m, 3H, H-9_{Neu}, H-6_{Neu}, and H-4_{Fuc}), 3.81 (dd, 1H, H-9_{Neu}), 3.76 (m, 1H, H-4_{Neu}), 3.63 (m, 2H, H-5_{Fuc} and H-3_{Fuc}), 2.41 (dd, 1H, $J_{gem} = 14.9$ Hz, $J_{3eq,4} = 10.5$ Hz, H-$3eq_{Neu}$), 2.64 and 2.19 (2s, 6H, 2Ac), 2.13 (dd, 1H, $J_{3ax,4} = 5.7$ Hz, H-$3ax_{Neu}$), 1.25 and 1.07 (2s, 6H, 2Me), 0.70 (d, 1H, $J_{5,6} = 6.3$ Hz, H-6_{Fuc}); ^{13}C NMR (125 MHz, $CDCl_3$): δ 169.8, 169.3, 167.5, 139.1, 138.7, 138.5, 136.2, 129.3, 128.9, 128.5, 128.4, 128.3, 128.1, 128.0, 127.8, 127.5, 127.4, 127.4, 108.9, 101.6, 86.8, 79.4, 77.6, 76.2, 75.3, 74.7, 73.5, 73.4, 71.3, 67.9, 63.5, 49.5, 38.9, 26.5, 26.0, 25.0, 21.1, 16.3.

3.18. Methyl (phenyl 4,7-di-*O*-acetyl-3,5-dideoxy-9-*O*-benzyl-2-thio-8-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-5-trifluoroacetamido-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (21)

To a solution of compound **13** (102 mg, 0.16 mmol) and compound **14** (83 mg, 0.19 mmol) in CH_2Cl_2 (5.3 mL), 4 Å molecular sieves (240 mg) was added. The suspension was stirred for 1 h and cooled to −80 °C. To the mixture, Cp_2ZrCl_2 (85 mg, 0.22 mmol) and AgOTf (57 mg, 0.22 mmol) were added, and stirring was continued for 3 h till TLC analysis (4:1, toluene/EtOAc) indicated the completion of the reaction. The reaction mixture was filtered through Celite. The combined filtrate and washings were extracted with $CHCl_3$, and the organic layer was washed with satd aq $NaHCO_3$ and water, dried over Na_2SO_4, and concentrated. The residue was purified with column chromatography on silica gel (4:1, *n*-Hex/EtOAc) to give **21α** (130 mg, 77%) and the corresponding β-isomer **21β** (8 mg, 5%).

21α; $[\alpha]_D$ −12.7 (*c* 1.6, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 7.56–7.22 (m, 25H, 5Ph), 6.50 (d, 1H, NH), 5.31 (dd, 1H, H-7), 5.18 (d, 1H, $J_{1,2} = 3.3$ Hz, H-1_{Fuc}), 5.02–4.94 (m, 2H, H-4_{Neu} and PhCH_2), 4.82–4.46 (m, 7H, 4PhCH_2), 4.21–4.07 (m, 4H, H-6_{Neu}, H-3_{Fuc}, H-$9'_{Neu}$, and H-5_{Fuc}), 4.04–3.97 (m, 2H, H-2_{Fuc} and H-8_{Neu}), 3.81 (q, 1H, H-5_{Neu}), 3.68 (d, 1H, H-4_{Fuc}), 3.59–3.54 (m, 4H, H-9_{Neu} and Me), 2.78 (dd, 1H, H-$3eq_{Neu}$), 2.05–1.85 (m, 7H, H-3ax $_{Neu}$, 2Ac), 1.09 (d, 1H, $J_{5,6} = 6.6$ Hz, H-6_{Fuc}); ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.9, 170.6, 169.7, 168.6, 139.3, 139.0, 138.3, 138.2, 137.1, 130.2, 129.0, 128.8, 128.6, 128.5, 128.5, 128.4, 128.3, 128.2, 128.0, 127.8, 127.7, 127.6, 127.5, 95.0, 86.9, 79.0, 78.1, 76.4, 75.2, 73.8, 73.5, 73.1, 73.0, 72.6, 69.8, 69.6, 67.1, 66.9, 53.0, 50.5, 38.1, 29.9, 21.0, 20.8, 17.1; MALDI-TOFMS *m*/*z* calcd for $C_{56}H_{60}F_3NO_{14}S$ $[M+Na]^+$: 1082.36. Found 1082.38.

3.19. Phenyl 4,7-di-*O*-acetyl-5-amino-9-*O*-benzyl-3,5-dideoxy-2-thio-8-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-D-*glycero*-α-D-*galacto*-2-nonulopyranoside-1,5-lactam (22)

With fucosyl fluoride donor **14**: To a solution of compound **12** (100 mg, 0.19 mmol) and compound **14** (100 mg, 0.23 mmol) in CH_2Cl_2 (5.3 mL), 4 Å molecular sieves (240 mg) was added. The suspension was stirred for 1 h and cooled to −80 °C. To the mixture, Cp_2ZrCl_2 (81 mg, 0.28 mmol) and AgOTf (71 mg, 0.28 mmol) were added, and stirring was continued for 44 h until TLC analysis (2:1, *n*-Hex/EtOAc) indicated the completion of the reaction. The reaction mixture was filtered through Celite. The combined filtrate and washings were extracted with $CHCl_3$, and the organic layer was washed with satd aq $NaHCO_3$ and water, dried over Na_2SO_4, and concentrated. The residue was purified with column chromatography on silica gel (2:1, *n*-Hex/EtOAc) to give **22α** (115 mg, 65%) and the corresponding β-isomer **22β** (38 mg, 21%).

With phenylthioglycoside donor **15**: To a solution of compound **12** (100 mg, 0.19 mmol) and compound **15** (153 mg, 0.29 mmol) in toluene (2.0 mL) 4 Å molecular sieves (240 mg) was added. The suspension was stirred for 1 h and cooled to −20 °C. To the mixture, NIS (79 mg, 0.35 mmol) and TfOH (3.1 μL, 35 μmol) were added and stirring was continued for 2.5 h. The reaction mixture was alkalized to pH 8 with satd aq $NaHCO_3$ at 0 °C and filtered through Celite. The combined filtrate and washings were extracted with $CHCl_3$, and the organic layer was washed with satd $Na_2S_2O_3$ and brine, dried over Na_2SO_4, and concentrated. The residue was purified with column chromatography on silica gel (2:1, *n*-Hex/EtOAc) to afford **22α** (133 mg, 74%) and **22β** (31 mg, 17%). **22α**: $[\alpha]_D$ −30.6 (*c* 0.5, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 7.52–7.24 (m, 25H, 5Ph), 6.74 (m, 1H, NH), 5.60 (dd, 1H, H-7_{Neu}), 5.05 (d, 1H, $J_{1,2} = 3.7$ Hz, H-1_{Fuc}), 4.95 (d, 1H, PhCH_2), 4.86 (m, 1H, H-4_{Neu}), 4.78–4.38 (m, 7H, PhCH_2), 4.21 (dd, 1H, H-6_{Neu}), 4.01–3.96 (m, 2H, H-5_{Fuc} and H-2_{Fuc}), 3.87 (m, 1H, H-5_{Neu}), 3.81 (m, 1H, H-8_{Neu}), 3.73–3.70 (m, 3H, H-3_{Fuc}, H-4_{Fuc}, and H-9_{Neu}), 3.51 (dd, 1H, H-$9'_{Neu}$), 2.42 (dd, 1H, H-$3ax_{Neu}$), 1.96–1.89 (m, 7H, H-$3eq_{Neu}$ and 2Ac), 1.08 (d, 1H, H-6_{Fuc}); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.2, 169.7, 168.5, 138.6, 138.5, 138.3, 137.3, 136.1, 128.9, 128.8, 128.6, 128.5, 128.4, 128.3, 128.2, 128.0, 127.7, 127.7, 127.6, 127.3, 97.4, 85.7, 78.4, 78.1, 75.7, 75.3, 74.8, 73.6, 73.0, 72.8, 72.0, 68.9, 68.5, 67.1, 49.7, 37.8, 29.6, 21.0, 20.9, 16.7; MALDI-TOFMS *m*/*z* calcd for $C_{53}H_{57}NO_{12}S$ $[M+Na]^+$: 954.35. Found 954.35. **22β**: $[\alpha]_D$ +37.4 (*c* 0.5, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$): δ 7.59–7.10 (m, 25H, 5Ph), 5.74 (m, 2H, NH and H-7_{Neu}), 4.82–4.73 (m, 5H, H-4_{Neu} and 2PhCH_2), 4.28 (d, 1H, $J_{1,2} = 7.6$ Hz, H-1_{Fuc}), 4.26 (m, 1H, H-8_{Neu}), 3.89 (dd, 1H, H-6_{Neu}), 3.83–3.71 (m, 4H, H-9_{Neu}, H-$9'_{Neu}$, H-5_{Neu}, and H-2_{Fuc}), 3.55 (m, 1H, H-4_{Fuc}), 3.43–3.39 (m, 2H, H-3_{Fuc}, H-5_{Fuc}), 2.39 (near t, 1H, $J_{gem} = 14.6$ Hz, $J_{3eq,4} = 10.4$ Hz, H-$3eq_{Neu}$), 2.07 and 157 (2s, 6H, 2Ac), 1.98 (dd, 1H, H-$3ax_{Neu}$), 1.12 (d, 3H, $J_{5,6} = 6.6$ Hz, H-6_{Fuc}); ^{13}C NMR (100 MHz, $CDCl_3$); δ 170.5, 169.4, 168.7, 138.8, 138.2, 138.1, 137.7, 136.1, 128.9, 128.8, 128.8, 128.6, 128.4, 128.4, 128.4, 128.2, 128.2, 128.1, 128.0, 127.7, 127.6, 127.6, 127.5, 127.3, 105.6, 105.5, 85.7, 82.8, 79.1, 78.4, 78.2, 78.1, 78.0, 75.2, 74.7, 74.4, 73.5, 73.2, 73.2, 73.1, 72.7, 70.4, 69.3, 69.0, 49.6, 49.5, 49.5, 37.6, 29.6, 21.3, 21.3, 21.2, 20.4, 20.3, 16.8; MALDI-TOFMS *m*/*z* calcd for $C_{53}H_{57}NO_{12}S$ $[M+Na]^+$: 954.35. Found 954.33.

3.20. Phenyl 4,7-di-*O*-acetyl-9-*O*-benzyl-5-benzyloxycarbamoyl-3,5-dideoxy-2-thio-8-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-D-*glycero*-α-D-*galacto*-2-nonulopyranoside-1,5-lactam (23)

To a solution of compound **22** (207 mg, 0.22 mmol) in pyridine (2.2 mL), CbzOSu (166 mg, 0.68 mmol) was added under an argon atmosphere. The suspension was stirred for 18 h at rt (TLC; 3:1, PhMe/EtOAc). After the quenching by the addition of CH_3OH, the mixture was co-evaporated with toluene. The residue was dissolved in $CHCl_3$, washed with 2 M HCl, water, satd aq $NaHCO_3$ and brine, dried (Na_2SO_4), and concentrated. The residue was purified by chromatography on silica gel (15:1, toluene/EtOAc) to give **23** (217 mg, 92%): $[\alpha]_D$ +2.0 (*c* 1.0, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$): δ 7.56–7.15 (m, 30H, 6Ph), 5.66 (dd, 1H, $J_{6,7} = 4.8$ Hz, $J_{7,8} = 9.0$ Hz, H-7_{Neu}), 5.25 and 5.21 (2d, 2H, $J_{gem} = 12.4$ Hz, PhCH_2), 5.08 (m, 1H, H-5_{Neu}), 5.01 (d, 1H, $J_{1,2} = 3.6$ Hz, H-1_{Fuc}), 4.98 (m, 1H, $J_{3eq,4} = 10.4$ Hz, $J_{3ax,4} = 5.8$ Hz, H-4_{Neu}), 4.93–4.32 (m, 8H, PhCH_2), 4.30 (near dd, 1H, H-6_{Neu}), 3.94 (dd, 1H, $J_{2,3} = 10.2$ Hz, H-2_{Fuc}), 3.88 (m, 2H, H-8_{Neu} and

H-5$_{Fuc}$), 3.82 (dd, 1H, $J_{3,4} = 2.6$ Hz, H-3$_{Fuc}$), 3.65 (m, 2H, H-4$_{Fuc}$, H-9$_{Neu}$), 3.45 (t, 1H, $J_{gem} = 10.2$ Hz, $J_{8,9'} = 10.2$ Hz, H-9$_{Neu}$), 2.52 (dd, 1H, $J_{gem} = 14.6$ Hz, H-3eq$_{Neu}$), 2.12 (dd, 1H, H-3ax$_{Neu}$), 1.99 and 1.92 (2s, 6H, 2Ac), 0.99 (d, 3H, $J_{5,6} = 6.6$ Hz, H-6$_{Fuc}$); ^{13}C NMR (100 MHz, CDCl$_3$): δ 170.7, 169.5, 164.9, 151.1, 138.9, 138.6, 138.5, 137.8, 137.3, 136.4, 134.6, 129.2, 129.1, 129.0, 128.9, 128.7, 128.6, 128.4, 128.2, 128.1, 128.0, 127.9, 127.8, 127.6, 127.5, 127.4, 127.3, 125.2, 97.5, 86.8, 78.8, 76.6, 75.7, 75.3, 74.8, 73.4, 73.0, 72.7, 72.2, 69.4, 67.9, 67.3, 67.0, 52.3, 37.3, 29.6, 25.4, 21.0, 20.7, 16.6; MALDI-TOFMS *m/z* calcd for $C_{61}H_{63}NO_{14}S$ [M+Na]$^+$: 1088.39. Found 1088.37.

3.21. Methyl [phenyl 4,7-di-*O*-acetyl-9-*O*-benzyl-5-benzyloxycarbamoyl-3,5-dideoxy-2-thio-8-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-D-*glycero*-α-D-*galacto*-2-nonulopyranosid]onate (24)

To compound **23** (23 mg, 21.5 μmol), a 10% Et$_3$N solution was added in CH$_3$CN/THF (3:1) (1.0 mL) and water (0.5 mL). The mixture was stirred for 50 h at 40 °C as monitored by TLC (15:1:0.1, CHCl$_3$/CH$_3$OH/AcOH). The mixture was diluted with EtOAc, washed with 2 M HCl and brine, dried (Na$_2$SO$_4$), evaporated, and dried in vacuo for 6 h. The crude material was dissolved in DMF (1.5 mL) under an argon atmosphere, cooled to 0 °C and CH$_3$I (44 μL, 0.71 mmol) and K$_2$CO$_3$ (146 mg, 1.06 mmol) were added to the solution. The mixture was stirred for 20 min at rt (TLC; 30:1, CHCl$_3$/CH$_3$OH), neutralized with IR-120 (H$^+$) and filtered through Celite. The combined filtrate and washings were concentrated and co-evaporated with toluene to remove DMF. The resulting syrup was purified by column chromatography on silica gel (150:1, CHCl$_3$/CH$_3$OH) to give **25** (89 mg, 92%); $[\alpha]_D$ −4.8 (*c* 1.0, CHCl$_3$); ^{1}H NMR (500 MHz, CDCl$_3$): δ 7.55–7.18 (m, 30H, 6Ph), 5.39 (dd, 1H, $J_{6,7} = 3.7$ Hz, H-7$_{Neu}$), 5.18 (d, 1H, $J_{1,2} = 3.4$ Hz, H-1$_{Fuc}$), 5.08–4.72 (6d, 6H, PhC*H*$_2$), 4.80 (td, 1H, $J_{3ax,4} = 11.9$ Hz, $J_{3eq,4} = 5.1$ Hz, $J_{4,5} = 11.2$ Hz, H-4$_{Neu}$), 4.66 and 4.60 (2d, 2H, PhC*H*$_2$), 4.51 (d, 1H, NH), 4.50 (2d, 2H, PhC*H*$_2$), 4.22 (m, 2H, H-5$_{Fuc}$ and H-8$_{Neu}$), 4.08 (dd, 1H, $J_{2,3} = 7.3$ Hz, H-3$_{Fuc}$), 3.99 (dd, 1H, H-2$_{Fuc}$), 3.95 (m, 2H, H-9$_{Neu}$ and H-6$_{Neu}$), 3.64 (m, 1H, H-4$_{Fuc}$), 3.59 (m, 4H, H-9$'_{Neu}$ and Me), 3.51 (near q, 1H, H-5$_{Neu}$), 2.73 (dd, 1H, $J_{gem} = 12.4$ Hz, H-3eq$_{Neu}$), 2.07 and 1.79 (2s, 6H, 2Ac), 1.83 (near t, 1H, H-3ax$_{Neu}$), 1.03 (d, 3H, $J_{5,6} = 6.3$ Hz, H-6$_{Fuc}$); ^{13}C NMR (100 MHz, CDCl$_3$): δ 170.31, 170.08, 168.38, 155.65, 139.18, 138.80, 138.75, 138.41, 136.67, 136.45, 129.82, 129.22, 128.94, 128.81, 128.74, 128.69, 128.36, 128.34, 128.23, 128.11, 128.08, 128.07, 127.96, 127.67, 127.60, 127.42, 127.39, 127.32, 127.26, 127.21, 127.16, 97.48, 87.13, 78.92, 77.80, 77.31, 76.59, 76.24, 75.98, 75.28, 74.72, 73.32, 72.91, 71.98, 70.62, 69.53, 69.09, 66.78, 66.74, 52.81, 51.71, 51.64, 37.92, 20.91, 20.56, 16.68; MALDI-TOFMS *m/z* calcd for $C_{62}H_{67}NO_{15}S$ [M+Na]$^+$: 1120.41. Found: 1120.42.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.carres.2006.03.017.

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