Divergent Strategy for the Synthesis of α2-3-Linked Sialo-oligosaccharide Libraries Using a Neu5TFA-(α2-3)-Gal Building Block

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Abstract: A novel Neu5TFA-(α2-3)-Gal building block with removable protecting group at N5′ was introduced and used in the divergent synthesis of a library of *N*-acetyl and *N*-glycolyl derivatives of oligosaccharides related to sialyl-(α2-3)-*N*-acetyl-lactosamine and sialyl-(α2-3)-*N*-acetyl-isolactosamine, with or without sulfate at O6 of glucosamine residue.

Key words: bioorganic chemistry, glycosides, glycosylation, protecting groups, sialic acids

Glycoprotein and glycolipid carbohydrate chains terminated by sialic acids are involved in a wide range of biological phenomena ranging from cell–cell adhesion and mobility to oncogenesis and recognition by viruses and bacteria.[1] Therefore, the synthesis and the biomedical investigation of sialic acid containing glycoconjugates, oligosaccharides, and their analogues is a very important area of research aiming at understanding their biological roles and determining their therapeutic relevance.

Sialic acid residues are introduced into an oligosaccharide by a glycosylation reaction called sialylation, which is by no means a trivial one.[2] Although a tremendous progress has been achieved in chemical sialylation,[2,3] the outcome of a particular sialylation can rarely be predicted, which makes optimization[4d] of almost every sialylation necessary. Many variables[2–4] may become important – the nature of protecting groups both on glycosyl donor and glycosyl acceptor,[5] method of activation,[6] concentration of reagents,[4b,d] and the presence of other compounds in the reaction mixture (including 'nonreacting' additives or impurities)[4a–c] to name a few. For this reason, it is not surprising that synthetic strategies, which minimize the number of sialylation steps by using common building blocks, are becoming increasingly popular for the synthesis of sialo-oligosaccharides.[7,8] The preparation of sialo-oligosaccharides becomes even more challenging when both naturally occurring *N*-acetyl and *N*-glycolyl (Gc) derivatives of the same oligosaccharide backbone and their analogues with diverse substituents at N5 of sialic acid residue are required. Although each form can be synthesized separately,[9] the use of sialyl donor with a suitable temporary protection at N5 is generally considered more reasonable.[2d,e] One of the practical approaches to libraries of sialo-oligosaccharides, which comprise α2-3 intersaccharidic linkage, with almost any N-substituent from the single precursor involves the use of a sialyl-(α2-3)-galactose [Neu-(α2-3)-Gal] building block with removable protecting group at the N5 of sialic acid residue.[7]

Herein we introduce a novel building block, Neu5TFA-(α2-3)-Gal, and use it in the divergent synthesis of *N*-acetyl and *N*-glycolyl derivatives of oligosaccharides related to sialyl-(α2-3)-*N*-acetyl-lactosamine and sialyl-(α2-3)-*N*-acetyl-isolactosamine, some of which carry sulfate at O6 of glucosamine residue. All oligosaccharides were synthesized as glycosides with the 3-aminopropyl spacer aglycon that allows their use for the preparation of neoglycoconjugates and glycoarray printing.[10]

The Neu5TFA-(α2-3)-Gal building block was designed as a per-O-acetylated glycosyl bromide **4** (Scheme 1) with *N*-trifluoroacetyl (TFA) temporary protecting group at N5 of sialic acid residue that can be readily removed by alkali treatment and then acylated with an appropriate reagent. For this reason, the amino group in the aglycon was generated at the later stages of synthesis from orthogonal azido group by catalytic hydrogenation.

The assembly of sialyl-(α2-3)-galactose building block by sialylation of an appropriate glycosyl acceptor was optimized earlier[4d] to give stereoselectively (α/β = 20:1) the disaccharide **1** as a 4-methoxyphenyl (MP) glycoside in 71% yield. Straightforward de-O-benzylation, O-acetylation (→ **2**, 93%) and oxidative removal of the MP aglycone followed by O-acetylation cleanly gave (Scheme 1) glycosyl acetate **3** (95% from **2**) as a mixture of anomers (α/β = ca. 1:1).[11] Glycosyl bromide **4** was readily obtained by treatment of glycosyl acetate **3** with $TiBr_4$[12] in CH_2Cl_2 and then directly used in glycosylations.[13]

In order to evaluate the glycosylation potential of the building block **4**, the latter was involved into glycosylation reaction with a primary alcohol, 3-chloropropanol (**5**), in the presence of AgOTf and *N*,*N*,*N*′,*N*′-tetramethylurea in CH_2Cl_2[14] analogously to the procedure described earlier for Neu5Ac-(α2-3)-Gal building block[8a] (Scheme 3). The reaction of **4** with excess **5** (4 equiv) cleanly gave the corresponding glycoside **14** in 80% yield.[15–17] Substi-

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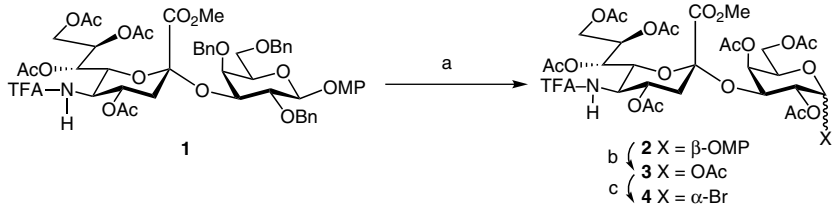
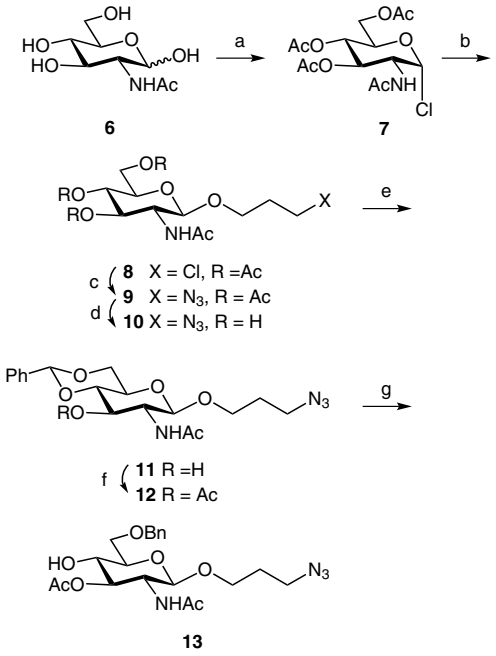


Scheme 1 Synthesis of Neu5TFA-(α2-3)-Gal glycosyl donor. *Reagents and conditions*: (a) (1) H_2, Pd/C, MeOH; (2) Ac_2O, pyridine (93%); (b) (1) $(NH_4)_2Ce(NO_3)_6$, MeCN–H_2O (5:1), 0 °C, 0.5 h; (2) Ac_2O, pyridine (95%); (c) $TiBr_4$, CH_2Cl_2, r.t., 3 h (quant.).

tution of chlorine with azide and deacylation under basic conditions[18] gave the corresponding 3-azidopropyl glycoside as 5′-amino derivative **15** in 85% yield. Acylation of the free amino group in **15** with $AcOCH_2CO_2C_6H_4NO_2$ in DMSO[19] followed by reduction of the azide by catalytic hydrogenation furnished *N*-glycolyl GM4 disaccharide as the spacered glycoside **16** in 55% yield.[20,21]



Scheme 2 Synthesis of GlcNHAc glycosyl acceptors. *Reagents and conditions*: (a) AcCl; (b) $Cl(CH_2)_3OH$ (**5**), $Cl(CH_2)_2Cl$; (c) NaN_3, DMSO, 80 °C; (d) 0.05 M MeONa, MeOH; (e) $PhCH(OMe)_2$, TsOH, MeCN (28% from **6**); (f) Ac_2O, pyridine; (g) $NaBH_3CN$, MsOH, THF (58% from **11**).

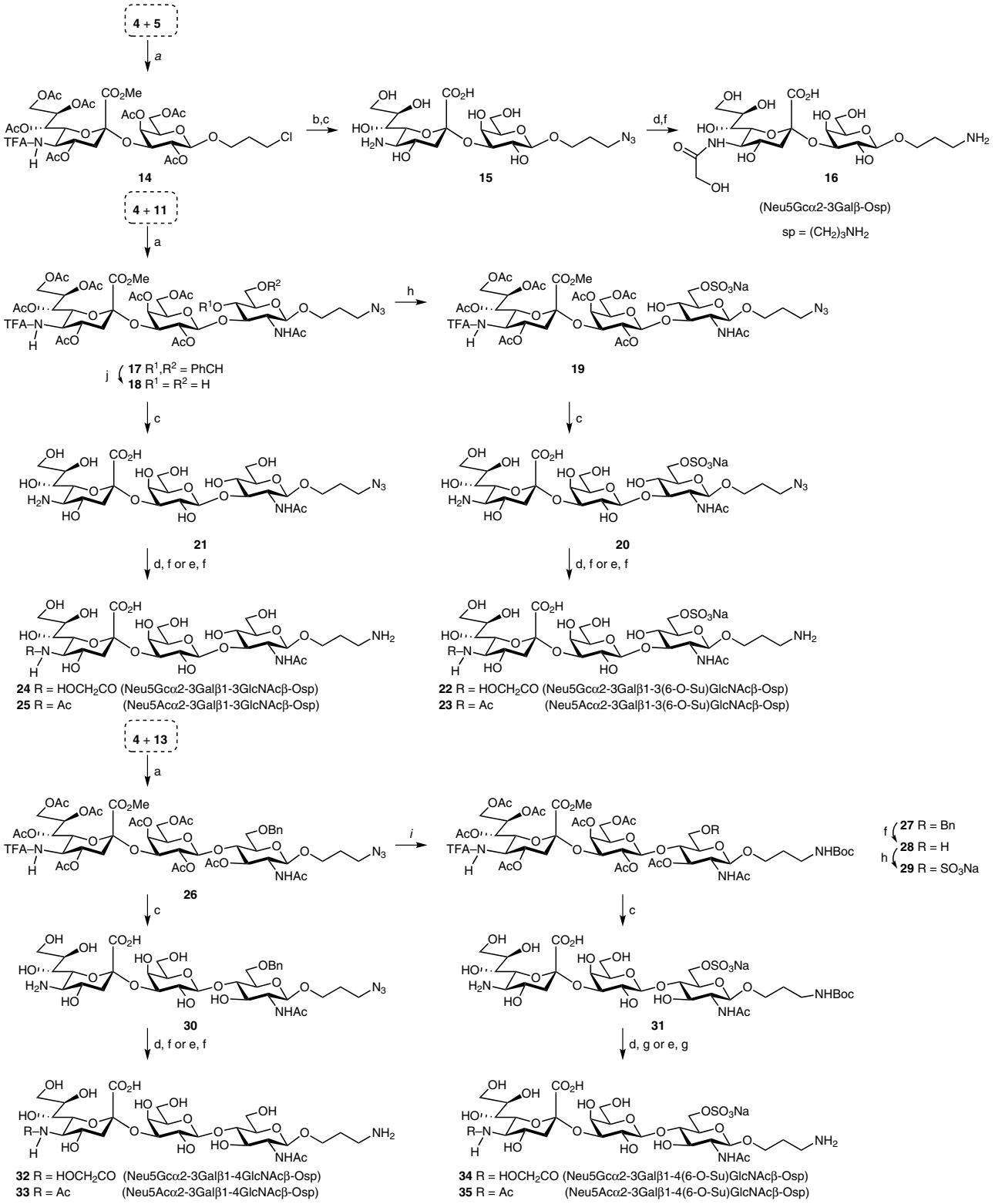
Glycosylation of glycosyl acceptors **11**[22] and **13** (Scheme 2)[23] with secondary hydroxy groups at C3 and C4, respectively, was performed with a slight excess (1.3 equiv) of glycosyl donor **4** analogously to the synthesis of glycoside **14** except for the different donor/acceptor ratio.[16] Initially formed trisaccharide **17** with *O*-benzylidene group without purification was treated with AcOH to give the corresponding diol **18**. The trisaccharides **18** and **26** were isolated in good yields (73% from **11** and 63% from **13**, respectively), which indicate efficiency of the proposed approach for the assembly of sialo-oligosaccharides.[15,25] Each oligosaccharide **18** and **26** obtained represents a branching point for the straightforward access to a set of derivatives, either with or without sulfate group at O6 of the glucosamine residue, differing in the nature of the acyl substituent at N5 of the sialic acid residue.

The procedure for N-acylation of N5 of the sialic acid residue in trisaccharides **18** and **26** essentially followed the one used for disaccharide **14**. Thus, all O-protecting groups were cleaved under basic conditions[18] to give the corresponding 5″-amino derivatives **21** and **30**, which were separately treated either with $AcOCH_2CO_2C_6H_4NO_2$ in DMSO[19] (N-glycolylation) or Ac_2O in MeOH (N-acetylation) followed by reduction of the azide by catalytic hydrogenation to give the target spacered trisaccharides in the following overall yields over three steps: **24** (50%), **25**[8a] (53%), **32**[9b] (73%), and **33**[8a,22] (83%).[20,21]

Selective sulfation of the primary hydroxy group of trisaccharide diol **18** with pyridine·SO_3 in pyridine[27] at low temperature lead to preferential formation of 6-O-sulfated (SO_3Na = Su) derivative **19**. Deprotection of **19** under basic conditions[18] gave the corresponding 5″-amino derivative **20**, which was transformed as described above to the target *N*-glycolyl- and *N*-acetyl-derivatives of spacered trisaccharides **22** and **23**[8a] in 69% and 79% yields, respectively.[28]

Hydroxy group at C6 of glucosamine residue to be sulfated was protected in trisaccharide **26** as *O*-benzyl ether that is also cleavable under conditions of catalytic hydrogenation used for reduction of azide in previous examples. Therefore, in this case, the azido group in the aglycon of **26** was first reduced with Ph_3P–H_2O to give the corresponding amine protected immediately as *N*-Boc derivative **27**, from which the *O*-benzyl group was removed by catalytic hydrogenolysis to furnish the *N*-Boc-protected 3-aminopropyl glycoside **28** in 75% yield. The released 6-OH group of the glucosamine residue in **28** was then sulfated with pyridine·SO_3 in pyridine[27] to give 6-*O*-Su derivative **29**. All O-protecting groups in **29** were cleaved under basic conditions[18] to give the 5″-amino derivative **31**, which was treated either with $AcOCH_2CO_2C_6H_4NO_2$ in DMSO[19] or Ac_2O in MeOH followed by removal of *N*-Boc protecting group from the aglycon by treatment with TFA[29] to give spacered trisaccharides **34** and **35**[8a] in 80% and 81% yields, respectively.[28]

The structures of all target compounds and key intermediates were confirmed by high-resolution NMR spectroscopy and mass spectrometry.[30,31] Spectroscopic data of *N*-acetyl-derivatives **23**, **25**, **33**, and **35** coincided with those described previously.[8a] ^1H NMR spectra of *N*-glycolyl de-



Scheme 3 Synthesis of a library of sialo-oligosaccharides. *Reagents and conditions*: (a) AgOTf, $Me_2NCONMe_2$, MS 4 Å, CH_2Cl_2, r.t., 20 h (80% of **14**, 63% of **26**); (b) NaN_3, DMSO, 40 °C, 5 d; (c) (1) 0.1 M MeONa–MeOH, 1 h; (2) 0.1 M aq NaOH, 16 h (85% of **15**); (d) (1) $AcOCH_2CO_2C_6H_4NO_2$, Et_3N, DMSO, 16 h; (2) 0.1 M aq NaOH, 1 h; (e) Ac_2O, MeOH, 0.5 h; (f) 10% Pd/C, H_2, MeOH–H_2O–NH_3 (1:1:0.005), 16 h (for **27**: MeOH, 2 h; 55% of **16** from **15**, 69% of **22** from **18**, 79% of **23** from **18**, 50% of **24** from **18**, 53% of **25** from **18**, 75% of **28** from **26**, 73% of **32** from **26**, 83% of **33** from **26**); (g) (1) TFA, 0.2 h; (2) 0.1 M aq NaOH, 0.5 h (80% of **34** from **28**, 81% of **35** from **28**); (h) pyridine·SO_3, py, 3 h (for **18**: –15 °C to –20 °C; for **28**: r.t.); (i) (1) Ph_3P, THF–H_2O (10:1), 16 h; (2) Boc_2O, 1 h; (j) 80% aq AcOH, 70 °C, 2 h (73% of **18** from **11**).

rivatives were very close to those of the respective *N*-acetyl derivatives. While the former exhibit a characteristic signal for the CH_2 group (s, 2 H, δ = ca. 4.1 ppm) of the *N*-glycolyl moiety, the latter exhibit the NAc signal (s, 3 H, δ = ca. 2.0 ppm).

In summary, we have introduced a novel sialyl-(α2-3)-galactose building block with removable protecting group at N5′ and used it for the straightforward synthesis of a library of sialo-oligosaccharides. Although here we prepared only natural *N*-acetyl and *N*-glycolyl derivatives, other acyl groups can be introduced to N5 of the sialic acid residue using this methodology. Due to its simplicity and robustness (related to the rational choice of protecting and leaving groups), the approach suggested may streamline the preparation of complex sialo-oligosaccharide libraries by researchers without specialized background in carbohydrate chemistry.

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Supporting Information for this article is available online at http://www.thieme-connect.com/ejournals/toc/synlett. Included are spectroscopic and analytical data for key building blocks and oligosaccharides synthesized.

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(11) Although the α- and β-anomers of **3** can easily be separated by column chromatorgaphy on silica gel (10 → 60% EtOAc in benzene) and crystallization (Me_2CO–hexanes), their mixture can be used in the preparation of glycosyl bromide **4**.

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(15) The glycosylation yields achieved with *N*-trifluoroacetyl glycosyl bromide **4** were comparable or slightly higher (5–11%) than those achieved earlier (ref. 8a) with the corresponding *N*-acetyl glycosyl bromide [Neu5Ac-(α2-3)-Gal building block], which was prepared from the parent glycosyl acetate by treatment with HBr in AcOH.

(16) **Typical Glycosylation Procedure**
A mixture of glycosyl acceptor (0.3 mmol), AgOTf (0.39 mmol), *N*,*N*,*N*′,*N*′-tetramethylurea (0.39 mmol), and freshly activated MS 4 Å (300 mg) in anhyd CH_2Cl_2 (7 mL) was stirred for 30 min at r.t. in the dark. Additional portion of MS 4 Å (100 mg) was added, and a solution of glycosyl bromide **4** (0.39 mmol) in anhyd CH_2Cl_2 (3 mL) was added. The mixture was stirred for 15–20 h and filtered, the filtrate was concentrated in vacuo, and the product was isolated by silica gel column chromatography.

(17) In this particular case different amounts of glycosyl donor **4** (0.3 mmol) and glycosyl acceptor **5** (1.2 mmol) were used, all other conditions were the same as described in the typical procedure (ref. 16).

(18) Deprotection of compounds **14**, **18**, **19**, **26**, and **29** under basic conditions involved treatment with MeONa in MeOH, followed by aq NaOH, neutralization with AcOH, and finally desalting by gel chromatography on Sephadex LH-20 (MeCN–H_2O, 1:1) gave compounds **15**, **20**, **21**, **30**, and **31** with the NH_2 group at C5 of the sialic acid residue and the latent amino group in the spacer aglycon (N_3 or NHBoc) in virtually quantitative yields.

(19) In order to remove *O*-acyl substituent in glycolyl fragment, the initially formed product was treated with aq NaOH, neutralized with AcOH, and only then was the product isolated by gel chromatography on Sephadex LH-20 (MeCN–H_2O, 1:1). .

(20) Compounds **16**, **24**, **25**, **32**, and **33** (internal salts) were isolated from the reaction mixtures by ion-exchange chromatography on Dowex 50W × 4 resin (H^+ form; elution with 1 M aq pyridine).

(21) Oligosaccharides **16**, **24**, and **25** were additionally purified by reversed-phase HPLC (Phenomenex Luna C18, 5 µm particle size, 100 Å pore size) by elution with H_2O.

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(23) Treatment of **6** with AcCl generated the corresponding acetylated glycosyl chloride **7**, which was further reacted with alcohol **5** to give the mixture of anomeric 3-chloropropyl glycosides **8**. The β-glycoside was separated by crystallization (EtOAc–Et_2O–hexanes) and chromatography of the mother liquor (silica gel, 5 → 30% Me_2CO in benzene), then chlorine in the aglycon was substituted with azide (→ **9**), and after de-O-acetylation (→ **10**) 4,6-*O*-benzylidene acetal was installed selectively to give the target 3-OH alcohol **11** in 28% overall yield. Acetylation of the single hydroxyl group in **11** followed by regioselective reductive ring opening of the benzylidene acetal in **12** with $NaBH_3CN$–MsOH in THF[24] gave the target 4-OH alcohol **13** in 58% overall yield.

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(28) Sulfated derivatives **22**, **23**, **34**, and **35** were isolated by ion-exchange chromatography on DEAE Sephadex A-25 (OAc^- form; elution with 1 M aq pyridine–AcOH, pH 6.5). After freeze-drying and ion-exchange on Dowex 50W × 4 resin (Na^+ form, elution with H_2O) the corresponding Na^+ salts were obtained.

(29) Treatment of the *N*-Boc derivatives of trisaccharides with TFA was accompanied by concomitant formation of the corresponding lactones, which were hydrolyzed with aq NaOH (excess NaOH was neutralized with AcOH) prior to isolation of the target spacered trisaccharides **34** and **35**.

(30) **Analytical Data of Sulfated Oligosaccharides**
Compound **22**: $[\alpha]_D^{25}$ –0.2 (*c* 0.42, MeCN–H_2O = 1:1). ^{1}H NMR (700 MHz, D_2O): δ = 1.82 (dd ≈ t, *J* = 12.1 Hz, 1 H, H-3_{ax}″), 1.99 (m, 2 H, CH_2 sp), 2.07 (s, 3 H, NCOMe), 2.81 (dd, $J_{3eq,3ax}$ = 12.5 Hz, $J_{3eq,4}$ = 4.6 Hz, 1 H, H-3_{eq}″), 3.142 (m ≈ t, *J* = 6.9 Hz, 2 H, NCH_2 sp), 3.57 (dd, $J_{1,2}$ = 7.9 Hz, $J_{2,3}$ = 9.8 Hz, 1 H, H-2′), 3.63 (m, 2 H, H-7″, H-4), 3.67 (dd, $J_{9a,9b}$ = 11.9 Hz, $J_{8,9b}$ = 6.1 Hz, 1 H, H-9b″), 3.70 (ddd ≈ dd, $J_{5,6a}$ = 3.9 Hz, $J_{5,6b}$ = 8.2 Hz, 1 H, H-5′), 3.73–3.93 (m, 10 H), 3.96 (dd ≈ t, *J* = 10.2 Hz, 1 H, H-5″), 3.97 (dd ≈ d, *J* = 3.1 Hz, 1 H, H-4′), 4.03 (m, 1 H, OCH sp), 4.12 (dd, $J_{2,3}$ = 9.8 Hz, $J_{3,4}$ = 3.1 Hz, 1 H, H-3′), 4.15 (s, 2 H, CH_2 Gc), 4.25 (dd, $J_{5,6b}$ = 5.9 Hz, $J_{6a,6b}$ = 11.3 Hz, 1 H, H-6b), 4.41 (dd, $J_{5,6a}$ = 1.9 Hz, $J_{6a,6b}$ = 11.3 Hz, 1 H, H-6a), 4.54 (d, $J_{1,2}$ = 7.9 Hz, 1 H, H-1′), 4.60 (d, $J_{1,2}$ = 8.5 Hz, 1 H, H-1). ^{13}C NMR (176 MHz, D_2O): δ = 22.4, 26.7, 37.8, 39.9, 51.4, 54.4, 61.1 (2 C), 62.5, 67.3, 67.3, 68.1, 68.2, 68.3, 68.6, 69.2, 71.9, 72.6, 73.3, 75.2, 75.7, 82.2, 99.7, 101.1, 103.5, 173.9, 174.8, 175.8. ESI-HRMS: *m/z* calcd for $C_{28}H_{48}N_3O_{23}S$ [M^-]: 826.2405; found: 826.2402.
Compound **34**: $[\alpha]_D^{25}$ –7 (*c* 0.46, MeCN–H_2O = 1:1). ^{1}H NMR (700 MHz, D_2O): δ = 1.80 (dd ≈ t, *J* = 12.1 Hz, 1 H, H-3_{ax}″), 1.94 (m, 2 H, CH_2 sp), 2.02 (s, 3 H, NCOMe), 2.75 (dd, $J_{3eq,3ax}$ = 12.4 Hz, $J_{3eq,4}$ = 4.6 Hz, 1 H, H-3_{eq}″), 3.10 (m ≈ t, *J* = 6.8 Hz, 2 H, NCH_2 sp), 3.54 (dd, $J_{1,2}$ = 7.9 Hz, $J_{2,3}$ = 9.8 Hz, 1 H, H-2′), 3.58 (dd, $J_{6,7}$ = 1.4 Hz, $J_{7,8}$ = 9.2 Hz, 1 H, H-7″), 3.63 (dd, $J_{8,9b}$ = 5.9 Hz, $J_{9a,9b}$ = 12.0 Hz, 1 H, H-9b″), 3.69–3.81 (m, 10 H), 3.87 (dd, $J_{9a,9b}$ = 12.1 Hz, $J_{8,9a}$ = 2.2 Hz, 1 H, H-9a″), 3.89–3.93 (m, 2 H, H-5″, H-8″), 3.95 (dd ≈ d, *J* = 2.9 Hz, 1 H, H-4′), 3.97 (m, 1 H, OCH sp), 4.10 (s, 3 H, CH_2 Gc), 4.11 (dd, $J_{2,3}$ = 9.9 Hz, $J_{3,4}$ = 3.0 Hz, 1 H, H-3′), 4.31 (dd, $J_{5,6a}$ = 4.4 Hz, $J_{6a.6b}$ = 11.2 Hz, 1 H, H-6a), 4.42 (dd ≈ d, *J* = 11.2 Hz, 1 H, H-6b), 4.53 (d, $J_{1,2}$ = 8.4 Hz, 1 H, H-1), 4.58 (d, $J_{1,2}$ = 7.8 Hz, 1 H, H-1′). ^{13}C NMR (176 MHz, D_2O): δ = 22.2, 26.7, 37.9, 39.7, 51.5, 55.1, 61.1, 61.1, 62.6, 66.4, 67.5, 68.1, 68.2, 68.3, 69.5, 71.6, 72.2, 72.6, 72.7, 75.2, 75.4, 77.5, 99.8, 101.4, 102.3, 174.0, 174.8, 175.8. ESI-HRMS: *m/z* calcd for $C_{28}H_{48}N_3O_{23}S$ [M^-]: 826.2405; found: 826.2402.

(31) For analytical data of other target oligosaccharides and key intermediates, see Supporting Information.

