Total Synthesis of Sialylated and Sulfated Oligosaccharide Chains from Respiratory Mucins

Jie Xia, James L. Alderfer, Conrad F. Piskorz, and Khushi L. Matta*[a]

Abstract: The total syntheses of several complex oligosaccharide moieties that occur in the core structure of sulfated mucins are reported. A trisaccharide acceptor was obtained through regio- and stereoselective sialylation of methyl (6-*O*-pivaloyl-β-D-galactopyanosyl)-(1$\rightarrow$3)-4,6-*O*-benzylidene-2-acetamido-2-deoxy-α-D-galactopyranoside with a novel sialyl donor. A tetrasaccharide, pentasaccharide, and hexasaccharide were constructed in predictable and controlled manner with high regio- and stereoselectivity after the successful preparation and employment of a disaccharide donor, trisaccharide donor, disaccharide acceptor, and trisaccharide acceptor building blocks. Finally, a mild oxidative cleaving method was adopted for the selective removal of 2-naphthylmethyl (NAP) in the presence of benzyl groups.

Keywords: glycosylations · oligosaccharides · protection groups · total synthesis

Introduction

In recent years, we have seen a tremendous amount of interest in the structure – activity relationship of the sulfated carbohydrate moieties which occur in O-linked mucinous glycoproteins, such as CF respiratory mucin,[1] colonic tumor associated glycoproteins,[2] and the natural ligands for selectin.[3] Studies on the biosynthesis of these glycoproteins and investigations of three enzymes [sulfotransferase, α(2,3)-sialyltransferase and α(1,3)-L-fucosyltransferase] involved in their assembly have likewise become subjects of great interest. As a result, the chemical synthesis of well defined oligosaccharides which are essential for the investigation of these enzymes is getting increased attention.[4] Consideration of the different reactivity of sugar ring hydroxyl groups in combination with detailed structural information obtainable from two dimensional NMR homonuclear (2D DQF-COSY, 2D ROESY, 2D TOCSY) and heteronuclear (HMQC or g-HSQC and HMBC) correlation experiments make it possible to develop a general strategy for regio- and stereoselective glycosylations that utilize unprotected or partially protected acceptors. The advancement of this approach overcomes the traditional, tedious multi-step protecting/deprotection reaction schemes, thereby providing a shorter and easier route for the synthesis of complex, biologically active oligosaccharide molecules.[5, 6] As illustrated in Figure 1, sulfate has been reported to be located at the C-6 position of GlcNAc in the Le^x moiety O-linked to the C-6 position of GalNAc. Synthesis of this type of structure requires a special protecting group which can be highly selectively removed in the presence of O-benzyl group. The 2-naphthylmethyl (NAP) group was recently introduced by Esko[7a] and Spencer[7b] as hydroxyl protection group in polyhydroxyl systems. This group can easily be cleaved by DDQ oxidation procedure. Herein, we describe a concise total synthesis of sialylated and sulfated oligosaccharides from respiratory mucins (Scheme1).

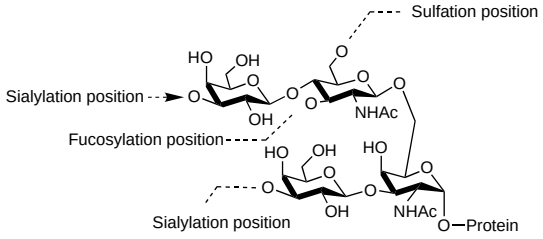

Figure 1. The core tetrasaccharide of O-linked glycoprotein.

Results and Discussion

Target oligosaccharides **1**–**3** were synthesized through the multiple use of the important intermediates: **11**, **13** (Scheme 2), **18** and **24** (Scheme 3). Disaccharide donor **11** and trisaccharide donor **13** were synthesized according to Scheme 2. Treatment of compound **5** with bis(tributyltin)-oxide[8] in refluxing toluene, followed by naphthyl methyl

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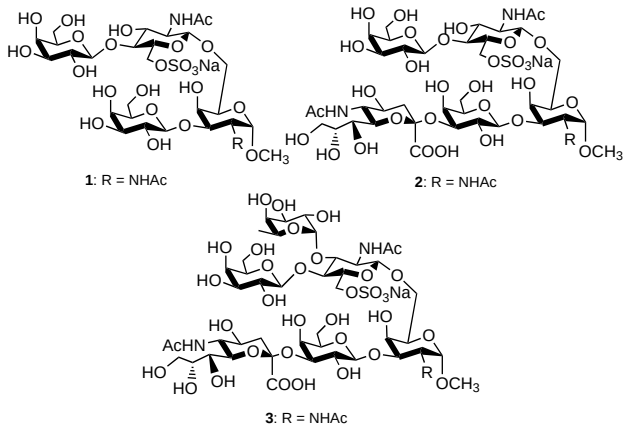
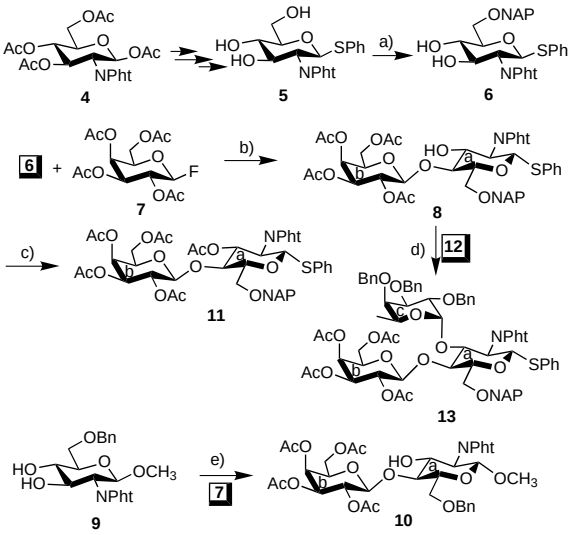


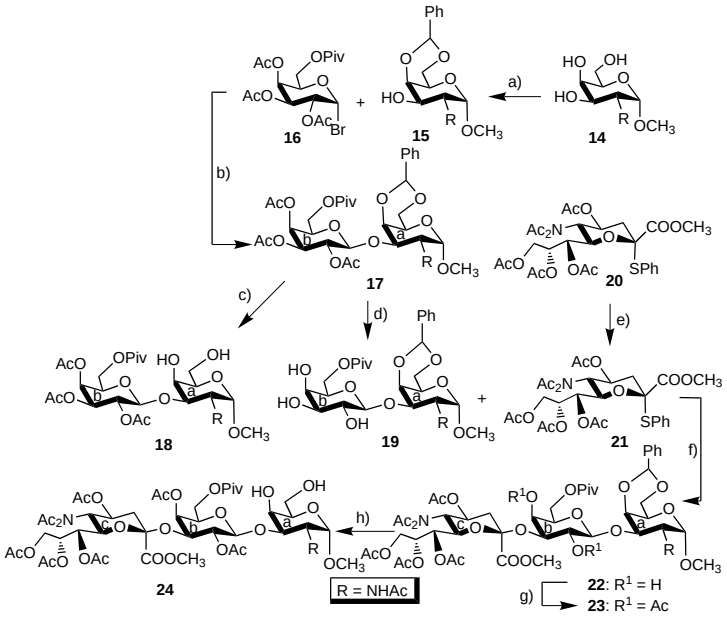
Scheme 1. Target structure of sialylated, sulfated oligosaccharides.



Scheme 2. a) nBu$_2$SnO/benzene, reflux, 4 h, Bu$_4$NBr, NAP-Br, 80 to 85 °C, 48 h, 69 %; b) SnCl$_2$/AgOTf, CH$_2$Cl$_2$/toluene, 4 Å MS, −15 to −5 °C, 12 h, 75 %; c) Ac$_2$O/pyridine 1:1, rt, 12 h, 79 %; d) CuBr$_2$$nBu_4$NBr/ClCH$_2$, CH$_2Cl_2$/DMF 5:1, 4 Å MS, rt, N$_2$, 16–24 h, 87 %; e) AgOTf/SnCl$_2$, CH$_2$Cl$_2$/toluene, 4 Å MS, −15 to −5 °C, 12 h, 73 %.

bromide in the presence of tetrabutylammonium iodide gave **6** in 69 % yield. Regioselective glycosylation of HO-4 of diol **6** with 2,3,4,6-tetra-*O*-acetyl-β-galactopyranosyl fluoride (**7**)[9] was successfully achieved using SnCl$_2$/AgOTf[10] catalyst, providing disaccharide **8** in good yield (75 %). Similar glycosylation of monosaccharide acceptor **9** with donor **7** afforded the $\beta(1\rightarrow4)$ linked disaccharide **10**. The structure of compound **10** was unambiguously established by a combination of 2D-NMR experiments (2D DQF-COSY, 2D ROESY) and X-ray structural analysis of disaccharide **10.**[11] Disaccharide **8** was acetylated with pyridine/Ac$_2$O 1:1 to give disaccharide donor **11** in good yield (79 %). A strong NOE cross peak between H^b-1 and H^a-4 of disaccharide **11** was indicative of a $\beta(1\rightarrow4)$ linkage. Disaccharide **8** was fucosylated with methyl 2,3,4-tri-*O*-benzyl-1-thio-β-L-fucoside (**12**)[12] catalyzed by CuBr$_2$/nBu$_4$NBr[13] to give **13** in excellent yield of 87 %.

The site of glycosylation, anomeric configuration of the newly formed bond, and stereochemistry of trisaccharide **13** were confirmed by complete assignment of all the peaks in the ^{1}H-NMR spectrum through a combination of 2D DQF-COSY and 2D-ROESY experiments. Compounds **18** and **24** were synthesized as depicted in Scheme 3. Thus, benzylidenation of



Scheme 3. a) PHCh(OCH$_3$)$_2$, *p*-TsOH, CH$_3$CN, rt, 12 h, 86 %; b) Hg(CN)$_2$, benzene/CH$_3$NO$_2$, 40 to 45 °C, 12 h, 70 %; c) 60 % HOAc, 60 to 65 °C, 1.5 h, 70 %; d) 1M CH$_3$ONa/CH$_3$OH, CH$_3$OH/CH$_2$Cl$_2$, −20 to −15 °C, 20 min; 81 %; e) isopryrenyl acetate/CAS, 65 °C, 16 h, quantitative; f) NIS/TfOH, CH$_2$Cl$_2$/CH$_3$CN 1:1, −45 to −40 °C, 12 h, 45 %; Ac$_2$O/pyridine 1:1, rt, 12 h; h) 60 % HOAc, 60 to 65 °C, 2 h, 76 %.

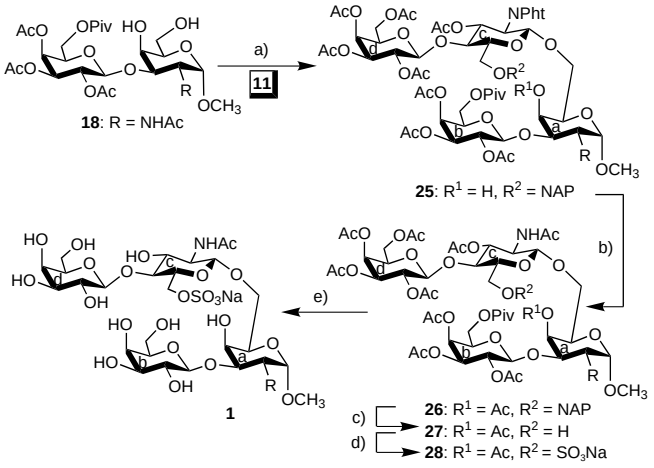
known compound **14**[14] with α,α-dimethoxytoluene in the presence of a catalytic amount of *p*-TsOH·H$_2$O afforded compound **15** in excellent yield (86 %). The $\beta(1\rightarrow3)$ linked disaccharide **17**, prepared according to well established procedure,[15] was treated with 60 % HOAc to give **18** in 70 % yield. Selective removal of the *O*-acetyl groups from **17** in the presence of the 6-*O*-pivaloyl group was successfully accomplished by treatment with sodium methoxide solution at −20 to −15 °C to give **19** in excellent yield (81 %). The next step employed the regio- and stereoselective sialylation of acceptor **19**. Synthesis of α-sialosides[16] has invariably been fraught with difficulties, largely because of the unique structural features of sialic acid. A number of approaches have been investigated in order to circumvent these difficulties.[17] To continue those efforts, we decided to explore the utility of a novel sialyl donor **21** with defined β-configuration as established by X-ray structure analysis.[11] It is noteworthy that the sialyl donor **21**, prepared from **20** in the presence of isopropenyl acetate and a catalytic amount of (±)-10-camphorsulfonic acid, was more reactive than **20**. The apparently more reactive HO-3 of galactose residue **b** of **19** was readily sialylated with **21** over both HO-2 and HO-4. Glycal, the by-product of β-elimination of **21**, was dramatically reduced by the trivial additional *N*-acetyl group which is consistent with the result observed by Boons and co-worker.[18] The $(2\rightarrow3)$-linkage of **22** was confirmed by observation of a weak NOE cross peak between H^b-3 of galactose residue **b** and H^c-3a of

sialic acid residue **c**,[19] and further confirmed by observation of a cross peak between C-2 of sialic acid residue **c** and H-3 of galactose residue **b** in HMBC spectrum. The α configuration of glycoside **22** was assigned according to literature methods.[20] This was further confirmed by observation of a strong cross peak between H-3a and C-1 of sialic acid residue in HMBC spectrum because α-sialoside has a larger heteronuclear coupling constant of $J_{C\text{-}1,H3a}$.[20b] After successful conversion of compound **19** into compound **22**, the acetylation of **22** with pyridine/Ac_2O 1:1 was performed in the presence of catalytic amounts of DMAP at room temperature to afford **23** which was then treated with 60% HOAc to give **24** (76% yield) in two steps.

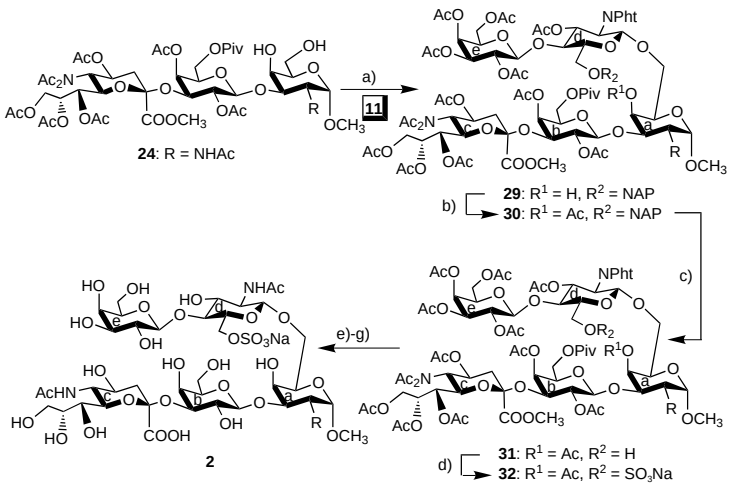
Target oligosaccharides **1**–**3** were then synthesized following Schemes 4, 5, and 6. Regioselective glycosylation of HO-6 of **18** with **11** was first attempted under controlled reaction conditions to give the $\beta(1\rightarrow6)$-linked **25** in excellent yield (89%). Similar, regioselective glycosylations of acceptor **24** with **11** and **13** were successfully performed under controlled conditions to give the $\beta(1\rightarrow6)$-linked **29** (59%) and **33** (79%), respectively. Linkage location and orientation was confirmed by observation of NOEs cross peaks between H-6a or H-6b of the *N*-acetylgalactosamine residue and H-1 of the *N*-phthalimido glucosamine residue in these three oligosaccharides by 2D-ROESY experiments. Therefore, **25**, **29**, and **33** were constructed in a predictable and controlled manner with high regio- and stereoselectivity. Tetrasaccharide **25** was treated with ethanol/NH_2-$NH_2\cdot H_2O$ 9:1, followed by pyridine/Ac_2O 1:1 in the presence of catalytic amounts of DMAP to give **26** in 83% yield in two steps. Compounds **29** and **33** were each treated with pyridine/Ac_2O 1:1 and catalytic amounts of DMAP to give acetylated **30** (quantitative yield) and **34** (85% yield). Similarity in the electron density between the NAP group and 4-methoxybenzyl (PMB)[21, 22] suggested that the former could be prone to mild oxidative cleavage. This was indeed the case, and compounds **27**, **31**, and **35** were readily obtained by treatment of **26**, **30**, and **34**, respectively with DDQ.

Noteworthy the removal of NAP from **34** requires carefully controlled conditions, since the tribenzyl fucose residue is known to be acid labile.[23] Conversion of **27** into **28** was accomplished by treatment of **27** with $SO_3\cdot$pyridine complex in dry pyridine. A similar procedure was applied for the conversion of **31** into **32**, and **35** into **36**. Sulfated tetrasaccharide **28** was treated with 1M sodium methoxide in methanol/water solution at room temperature to give **1**.

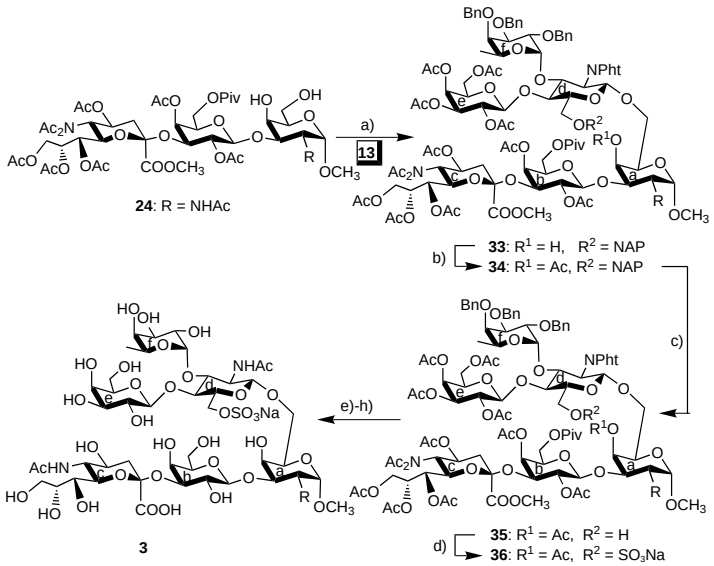
The deprotection procedure used for the conversion of **32** into **2** included three steps: a) removal of methyl from the carboxyl group with LiI in refluxing pyridine under N_2 atmosphere; b) removal of *N*-phthalimido with methanol/



Scheme 4. a) NIS/TfOH (cat.) 4 Å MS, CH_2Cl_2, -65 to $-60\,^{\circ}C$, 1 h, 89%; b) NH_2-$NH_2\cdot H_2O$/EtOH 9:1 80 to 85 °C; 2 h, then, Ac_2O/pyridine 1:1, rt, 12 h, 83%; c) DDQ, CH_2Cl_2/CH_3OH 4:1, rt, 18–24 h, 75%; d) SO_3/pyridine, pyridine, 0 to 5 °C, 9–12 h, 81%; e) 1M CH_3ONa/CH_3OH, CH_3OH, rt, 24 h, then Na^+-resin, rt, 4–5 h, 38%.



Scheme 5. a) NIS/TfOH, CH_2Cl_2, 4 Å MS, -65 to $-60\,^{\circ}C$, 2 h, 59%; b) Ac_2O/pyridine, DMAP, rt, in quantitative yield; c) DDQ, CH_2Cl_2/CH_3OH 4:1, rt, 18 h; d) $SO_3\cdot$pyridine, pyridine, 0 to 5 °C, 6 h, then, Na^+-resin, rt, 4 h, yield for c) to d): 63%; e) LiI/pyridine, 120 to 125 °C, 6–8 h; f) NH_2-$NH_2\cdot H_2O$/CH_3OH 1:5, 80 to 85 °C, then, Ac_2O/pyridine 1:1, rt, 12 h, 59% for two steps; g) 1M CH_3ONa/CH_3OH, rt, 12 h, 36% in three steps.



Scheme 6. a) NIS-TfOH/CH_2Cl_2, 4 Å MS, -65 to $-60\,^{\circ}C$, 2 h, 79%; b) Ac_2O/pyridine, DMAP, rt, 85%; c) DDQ, CH_2Cl_2/CH_3OH 4:1, rt, 12 h; 80% d) $SO_3\cdot$pyridine, pyridine, 0 to 5 °C, 6 h, 86%, then, Na^+-resin, rt, 4 h, e) LiI/pyridine, 120 to 125 °C, 6–8 h; f) NH_2-$NH_2\cdot CH_3OH$ 1:5, 80 to 85 °C, then, Ac_2O/pyridine 1:1, rt, 12 h, g) 1M CH_3ONa/CH_3OH, CH_3OH, rt, 12 h, h) 10% Pd/C, CH_3OH/HOAc 1:1, H_2, 12 h, 35% in four steps.

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NH_2-$NH_2 \cdot H_2O$ 5:1, followed by pyridine/Ac_2O 1:1 in the presence of catalytic amounts of DMAP; c) O-deacetylation with 1M sodium methoxide in metshanol/water solution at room temperature to give **2** (Scheme 5). Sulfated compound **36** was treated exactly as described for **32** to give **2** to furnish **3** (Scheme 6). The structures of **1–3** were fully characterized by ^{1}H homonuclear correlation NMR spectroscopy (2D DQF-COSY, 2D ROESY, 2D TOCSY), ^{13}C-NMR experiments and FAB-MS. The positive ion mode FAB mass spectrum of tetrasaccharide **1** {$C_{29}H_{49}O_{24}N_2SNa_2$: 887.3 $[M+Na]^+$} showed a pseudomolecular ion at m/z: 887.4. Scalar coupled networks of residues **a–d** of sodium sulfated tetrasaccharide **1** were identified by 2D DQF-COSY and TOCSY NMR spectra. Figure 2 shows the 1D spectrum of tetrasaccharide **1** in which three relatively strong signals and a weak signal are indicated for the anomeric region.

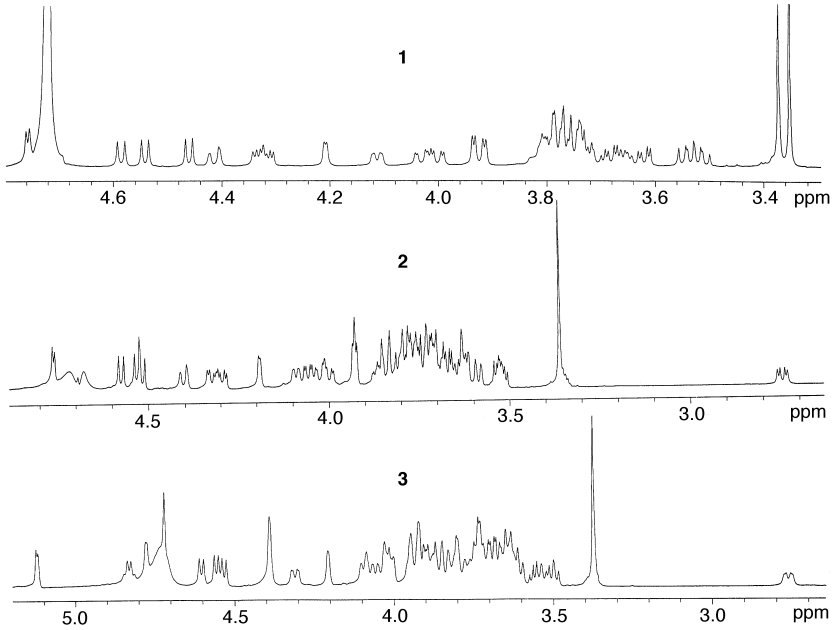

Figure 2. 600 MHz 1D ^{1}H-NMR spectra of **1** (D_2O+CD_3OD), **2** (D_2O), and **3** (D_2O) at 303.0 K.

An anomeric resonance of residue **a** at $\delta = 4.76-4.75$ was determined to be H^a-1 of the *N*-acetylgalactosamine residue **a** because of its strong NOE connectivity with the methyl group at $\delta = 3.37$. The coupling constant $J_{1,2} = 3.2$ Hz suggests that *N*-acetylgalactosamine has an α-configuration. Complete assignment of signals of residue **a** were carried out by analyses of the 2D DQF-COSY, 2D TOCSY and 2D ROESY NMR spectra. The anomeric signal at $\delta = 4.59-4.58$ was assigned to *N*-acetylglucosamine based on its coupling constant ($^3J_{1,2} =$ 8.4 Hz), and chemical shift value. Anomeric resonance of residue **b** and **d** at $\delta = 4.47-4.45$ and $4.55-4.53$ were considered to be due to galactose because they show weak spin connectivity between H-4 and H-5 in the 2D TOCSY and 2D DQF-COSY spectra, which are characteristic features.[24] Anomeric signals designated for residues **b** and **d** gave well solved doublets with the larger coupling constant ($^3J_{1,2} =$ 7.8 Hz) attributed to β-galactopyranoside configuration (e.g. $J_{1,2} = \approx 8.0$ Hz for β-D-Galp).[24, 25] Anomeric resonance at $\delta =$ 4.47–4.45 was attributed to H^b-1 of residue **b** due to the observation of a strong NOE cross peak between this signal and the signal at $\delta = 4.01-3.99$ (H^a-3). The positive ion-mode FAB mass spectrum of pentasaccharide **2** {$C_{40}H_{66}O_{32}N_3SNa_2$: 1178.6 $[M+Na]^+$} revealed a characteristic fragment ion at m/z: 877.4 $[M+Na-Neu5Ac]^+$. The positive ion-mode FAB mass spectrum of hexasaccharide **3** {$C_{46}H_{76}O_{36}N_3SNa_2$: 1324.5 $[M+Na]^+$} gave a pseudo-molecular ion at m/z: 1324.9. The position connectivity of pentasaccharide **2** and hexasaccharide **3** were confirmed by analyses of 2D ROESY spectrum.

Conclusion

We described an efficient route for the synthesis of sialylated and sulfated oligosaccharides from respiratory mucins based on the finding that the newly introduced electron-rich protecting group, the 2-naphthylmethyl (NAP) can readily be cleaved with DDQ in a manner analogous to that adopted for the 4-methoxybenzyl group. The *N*,*N*-diacetylamino sialic acid derivative **21** was the sialyl donor of choice because of its enhanced reactivity as compared with its parent acetamido compound **20**.

Experimental Section

General procedures: TLC was conducted on glass plates, precoated with 0.25 mm layer of silica gel 60 F-254 (Analtech GHLF uniplates); the components were visualized either by exposure to UV light or by spraying with a solution of 10% H_2SO_4 in ethanol, containing 0.2% *p*-anisaldehyde. Solutions were concentrated under reduced pressure. The silica gel used for column chromatography was Baker Analyzed (60–200 mesh). Optical rotations were measured at 25 °C with a Perkin–Elmer 241 polarimeter. $[\alpha]_D$ values are given in 10^{-1} deg cm^2 g^{-1}. ^{1}H-NMR spectra were recorded at 303 K with either a Bruker AM-400 or AMX-600 spectrometer. The values of δ [ppm] are given relative to the signal for internal Me_4Si ($\delta = 0$) for solutions in $CDCl_3$, CD_2Cl_2, CD_3OD, and D_2O. ^{13}C-NMR spectra were recorded at 303 K with a Bruker AM-400 (100.6 MHz) spectrometer using the signals for $CDCl_3$ ($\delta = 77.0$), CD_2Cl_2 ($\delta = 54.15$), CD_3OD ($\delta = 49.15$), [D_6]acetone ($\delta = 206.0$ or 29.8) as references. First-order chemical shifts and coupling constants (*J*/ Hz) were obtained from one-dimensional spectra and assignments of proton resonance were based on 2D DQF-COSY, 2D ROESY, and 2D TOCSY. Two-dimensional double-quantum filtered phase sensitive ^{1}H–^{1}H correlated spectra (2D DQF ^{1}H–^{1}H COSY), rotating-frame nuclear Overhauser enhancement spectroscopy (2D ROESY) and total correlation spectroscopy (2D TOCSY) were recorded at 303.0 K with a Bruker AM-400 (400 MHz) spectrometer and a Bruker AMX-600 (600 MHz) spectrometer. For ROESY experiments, the mixing time was set at 400 ms, for the TOCSY experiments 50 ms. ^{13}C–^{1}H heteronuclear multiple-bond correlation (HMBC) experiment was recorded at 303.0 K with a Bruker AMX-600 MHz spectrometer. All samples submitted for elemental analyses were dried under vacuum over P_2O_5 at room temperature. Elemental analyses were carried out by Robertson Microlit Laboratory, Madison, New Jersey. *p*-Toluene sulfonic acid monohydrate (*p*-TsOH$\cdot H_2O$) was co-evaporated

three times with dry acetonitrile at 80 °C before use. Dichloromethane, acetonitrile, methanol, benzene, and DMF were kept dry over 4 Å MS, pyridine was redistilled over potassium hydroxide, nitromethane was freshly distilled over P_2O_5.

Phenyl 6-*O*-naphthylmethyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (6): Bis(tributyltin) oxide (33.0 g, 54.86 mmol) was added to a solution of compound **5** (20.0 g, 49.88 mmol) in dry toluene (430 mL), and the mixture was heated until toluene (200 mL) had been distilled off. The temperature was then adjusted to 80 to 85 °C, and tetrabutylammonium iodide (20.26 g, 54.86 mmol) and 2-(bromomethyl)naphthalene (12.12 g, 54.86 mmol) were added and the stirring was continued at the same temperature for 48 h. The mixture was concentrated under reduced pressure, and the crude residue was applied to a column of silica gel and eluted with hexane/ethyl acetate 4:1, and then with CH_2Cl_2/MeOH 20:1 to give compound **6** (18.6 g, 69 %) as an amorphous solid. $R_f = 0.68$ (CH_2Cl_2/CH_3OH 20:1); ^{1}H NMR (CD_2Cl_2, 400 MHz): $\delta = 7.85 - 7.75$ (m, 4H; ArH), 7.37 – 7.35 (m, 5H; ArH), 7.25 – 7.24 (m, 7H; ArH), 5.63 (d, $J_{1,2} = 10.4$ Hz, 1H; H-1), 4.69 (d, $J_{gem} = 12.4$ Hz, 1H; $OCHC_{10}H_7$, ABq), 4.57 (d, $J_{gem} = 12.6$ Hz, 1H; $OCHC_{10}H_7$, ABq), 4.25 (t, $J = 8.4$ Hz, 1H; H-3), 4.14 (t, $J = 10.8$ Hz, 1H; H-2), 3.89 (dd, $J = 11.6$, 3.6 Hz, 1H; H-6a), 3.81 (dd, $J = 11.6$, 3.6 Hz, 1H; H-6b), 3.54 – 3.52 (m, 2H, H-4; H-5); ^{13}C NMR (CD_2Cl_2, 100.6 MHz): δ = 135.49, 134.27, 133.41, 133.16, 132.66, 132.32, 133.22, 131.72, 129.01, 128.91, 128.35, 128.10, 127.84, 126.63, 126.25, 126.03, 125.87, 83.73 (C-1), 78.96, 73.79, 72.92, 72.72, 70.10, 55.79; elemental analysis calcd (%) for $C_{31}H_{27}O_6NS$: C 68.74, H 5.02, N 2.59, S 5.92; found C 68.61, H 5.11, N 2.37, S 6.08.

Glycosylation procedure A: **With $SnCl_2$/AgOTf catalyst**: A solution of compound **6** or **9** (1 mmol), compound **7** (1.2 mmol), $SnCl_2$ (1.8 mmol) in dry dichloromethane/toluene 5:1 containing 4 Å MS (10 g per 100 mL for **6**, 8 g per 43 mL for **9**) was stirred at −15 °C for 1.5 – 2 h under N_2 atmosphere. AgOTf (1.8 mmol) was added. The mixture was stirred overnight at −15 to 0 °C under N_2 atmosphere then neutralized with triethylamine. Solids were filtered off and the organic layer washed with saturated $NaHCO_3$ solution, water, and dried (Na_2SO_4). The solvent was removed under reduced pressure to yield a crude mixture, which was then purified by passage through a silica gel column eluted with hexane/ethyl acetate 1:1 to give desired product.

Phenyl (2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1 → 4)-6-*O*-naphthylmethyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (8): Yield: 3.48 g, 75 % as an amorphous solid from **6**. $R_f = 0.54$ (hexane/ethyl acetate 1:1); ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 7.86 - 7.80$ (m, 6H; ArH), 7.68 – 7.64 (m, 2H; ArH), 7.52 – 7.40 (m, 5H; ArH), 7.28 – 7.16 (m, 3H; ArH), 5.64 (d, $J_{1,2} = 10.4$ Hz, 1H; H^a-1), 5.32 (d, $J = 2.0$ Hz, 1H; H^b-4), 5.18 (t, $J = 10.0$, 8.8 Hz, 1H; H^b-2), 4.92 (dd, 1H; H^b-3), 4.88 (d, J = 12.0 Hz, 1H; $OCH_AC_{10}H_7$, ABq), 4.71 (d, $J = 12.0$ Hz, 1H; $OCH_BC_{10}H_7$, ABq), 4.52 (d, $J = 8.0$ Hz, 1H), 4.45 (t, 1H), 4.05 – 4.03 (m, 4H), 3.87 – 3.71 (m, 4H), 2.10 (s, 3H; Ac), 1.96 (s, 3H; Ac), 1.94 (2s, 6H; 2Ac); ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ = 178.50 (C=O), 170.50 (C=O), 170.00 (C=O), 169.50 (C=O), 169.20 (C=O), 134.50, 132.75, 129.00, 128.50, 128.20, 128.00, 127.80, 126.58, 126.30, 126.00, 123.58, 123.50, 100.50, 83.50, 81.00, 79.20, 72.00, 70.80, 70.20 (2C), 69.50, 69.00, 68.50, 60.50, 58.50, 20.68 (Ac), 20.62 (Ac), 20.35 (Ac), 20.40 (Ac); elemental analysis calcd (%) for $C_{45}H_{45}O_{15}NS$: C 61.99, H 5.20, N 1.61, S 3.68; found C 61.90, H 5.11, N 1.80, S 3.70.

Methyl (2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1 → 4)-6-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (10): Yield: 2.21 g, 73 % as an amorphous solid from **9**. $R_f = 0.34$ (hexane/EtOAc 1:1); ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 7.84 - 7.82$ (m, 2H; ArH), 7.72 – 7.70 (m, 2H; ArH), 7.41 – 7.32 (m, 5H; ArH), 5.33 (d, $J = 3.2$ Hz, 1H; H^b-4), 5.19 (dd, $J = 8.0$, 10.4 Hz, 1H; H^b-2), 5.12 (d, $J_{1,2} = 8.8$ Hz, 1H; H^a-1), 4.94 (dd, $J = 3.6$, 10.2 Hz, 1H; H^b-3), 4.76 (d, $J_{gem} = 12.4$ Hz, 1H; OCHPh, ABq), 4.54 (d, $J_{gem} = 12.0$ Hz, 1H; OCHPh, ABq), 4.49 (d, $J_{1,2} = 8.0$ Hz, 1H; H^b-1), 4.40 (t, 1H; H^a-3), 4.16 (dd, $J = 8.8$ Hz, 1H; H^a-2), 4.06 – 4.04 (m, 2H; H^b-6b, H^b-6a), 3.95 (s, 1H; OH^a-3), 3.90 (t, $J = 7.2$, 6.4 Hz, 1H; H^b-5), 3.77 – 3.69 (m, 3H; H^a-5, H^a-4, H^a-6b), 3.65 – 3.62 (m, 1H; H^a-6a), 3.44 (s, 3H; OCH_3), 2.11 (s, 3H; Ac), 2.00 (s, 3H; Ac), 1.95 (s, 3H; Ac), 1.91 (s, 3H; Ac); ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ = 170.46 (C=O), 170.06 (C=O), 169.92 (C=O), 169.15 (C=O), 138.18, 133.99, 131.92, 128.55, 127.91, 127.86, 123.34, 101.54, 99.26, 82.06, 74.27, 73.74, 71.22, 70.81, 69.69, 68.80, 67.97, 66.90, 61.47, 56.75, 55.94, 20.72 (Ac), 20.56 (Ac), 20.50 (Ac), 20.33 (Ac); elemental analysis calcd (%) for $C_{36}H_{41}O_{16}N$: C 58.12, H 5.56, N 1.88; found C 58.00, H 5.59, N 1.81.

Phenyl (2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1 → 4)-3-*O*-acetyl-6-*O*-naphthylmethyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (11): Ac_2O (5 mL) was added to a solution of compound **8** (900 mg, 0.99 mmol) and DMAP (10 mg) in dry pyridine (5 mL). The mixture was stirred overnight at room temperature then concentrated under reduced pressure to a crude residue, which was applied to a short column of silica gel and eluted with hexane/ethyl acetate 1:1 to give compound **11** (741 mg, 79 %) as an amorphous solid. $R_f = 0.45$ (hexane/ethyl acetate 1:1); ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 7.96 - 7.25$ (m, 16H; ArH), 5.80 – 5.75 (m, 2H, $J_{1,2}$ = 10.0 Hz; H^a-1, $J = 10.0$, 8.4 Hz; H^a-3), 5.28 (d, $J = 3.2$ Hz, 1H; H^b-4), 5.05 (dd, $J = 7.6$, 10.4 Hz, 1H; H^b-2), 4.97 (d, $J_{gem} = 12.8$ Hz, 1H; $OCH_AC_{10}H_7$, ABq), 4.90 (dd, $J = 3.2$, 10.4 Hz, 1H; H^b-3), 4.75 (d, $J_{gem} = 11.2$ Hz, 1H; $OCH_BC_{10}H_7$, ABq), 4.59 (d, $J_{1,2} = 7.6$ Hz, 1H; H^b-1), 4.37 (t, $J = 10.4$ Hz, 1H; H^a-2), 4.09 – 4.03 (m, 3H; H^a-4, H^b-6a, H^b-6b), 3.89 – 3.87 (m, 2H; H^a-6a, H^a-6b), 3.76 – 3.74 (m, 1H; H^a-5), 3.63 (t, 1H; H^b-5), 2.12 (s, 3H; Ac), 2.05 (s, 3H; Ac), 1.98 (s, 3H; Ac), 1.91 (s, 3H; Ac), 1.90 (s, 3H; Ac); ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ = 170.28 (C=O), 170.19 (C=O), 170.01 (C=O), 169.99 (C=O), 168.91 (C=O), 167.85 (C=O), 167.32 (C=O), 135.44, 134.53, 134.32, 133.19, 133.08, 131.81, 131.66, 131.34, 129.03, 128.61, 128.27, 127.98, 127.92, 126.93, 126.86, 126.42, 126.18, 126.11, 126.06, 123.78, 123.63, 100.55 (C^b-1), 83.29 (C^a-1), 79.07, 75.48, 73.90, 72.12, 71.18, 70.57, 69.26, 66.91, 60.92, 60.40, 54.13, 21.68 (Ac), 20.64 (Ac), 20.60 (Ac), 20.57 (Ac); elemental analysis calcd (%) for $C_{47}H_{47}O_{16}NS$: C 61.63, H 5.39, N 1.53, S 3.50; found C 61.69, H 5.11, N 1.37, S 3.60.

Phenyl (2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1 → 4)-[(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-(1 → 3)]-6-*O*-naphthylmethyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (13): A solution of compound **8** (1.22 g, 1.40 mmol), methyl 2,3,4-tri-*O*-benzyl-1-thio-β-L-fucopyranoside (**12**, 2.62 g, 5.6 mmol), tetrabutylammonium bromide (1.81 g, 5.6 mmol) in dry 1,2-dichloroethane/DMF (20 mL, 5:1) containing 4 Å MS (8 g) was stirred at room temperature for 2 h under N_2 atmosphere. $CuBr_2$ (1.25 g, 5.6 mmol) was then added and stirring continued for overnight. Additional portions of compound **12** (1.31 g) and $CuBr_2$ (750 mg) were added and stirring was continued for a total of 18 h. The solids were filtered off and the organic layer was washed with sat. $NaHCO_3$ solution, water, dried (Na_2SO_4), and concentrated under reduced pressure. The crude product was applied to a column of silica gel eluted with hexane/ethyl acetate 2:1 to give compound **13** (1.58 g, 1.22 mmol, 87 %) as a white solid. $R_f = 0.17$ (hexane/ethyl acetate 2:1); ^{1}H NMR ($CDCl_3$, 600 MHz): $\delta = 7.90 - 7.82$ (m, 4H; ArH), 7.72 – 7.67 (m, 4H; ArH), 7.50 – 7.44 (m, 3H; ArH), 7.37 – 7.35 (m, 2H; ArH), 7.26 – 7.01 (m, 18H; ArH), 5.48 (d, $J = 10.4$ Hz, 1H; H^a-1), 5.15 (d, $J = 2.8$ Hz, 1H; H^b-4), 5.00 (dd, $J = 10.4$, 10.0 Hz, 1H; H^b-2), 4.94 (d, $J_{gem} = 12.4$ Hz, 1H; $OCH_AC_{10}H_7$, ABq), 4.83 – 4.69 (m, 5H; H^b-3, H^c-1, H^b-1, H^a-3, OCHPh, ABq), 4.66 (d, $J_{gem} = 12.4$ Hz, 1H; $OCH_BC_{10}H_7$, ABq), 4.61 – 4.49 (m, 5H; H^c-5, OCHPh, OCHPh, OCHPh, ABq, H^a-2), 4.41 (d, $J_{gem} = 12.0$ Hz, 1H; OCH_APh, ABq), 4.24 (d, $J_{gem} = 12.0$ Hz, 1H; OCHPh, ABq), 4.17 (t, $J = 10.0$, 8.8 Hz, 1H; H^a-4), 4.06 (dd, 1H; H^b-6b), 3.87 – 3.82 (m, 4H; H^a-6b, H^a-6a, H^b-6a, H^c-3), 3.77 (dd, 1H; H^c-4), 3.58 – 3.55 (m, 2H; H^c-2, H^a-5), 3.43 – 3.39 (m, 1H; H^b-5), 1.92 (s, 3H; Ac), 1.91 (s, 3H; Ac), 1.87 (s, 3H; Ac), 1.79 (s, 3H; Ac), 1.15 (d, $J = 6.4$ Hz, 3H; CH_3^c); ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ = 170.09 (C=O), 169.94 (C=O), 168.89 (C=O), 138.84, 138.35, 135.49, 134.42, 133.43, 133.25, 132.65, 131.89, 129.04, 128.70, 128.38, 128.34, 128.27, 128.19, 128.15, 128.09, 128.03, 127.99, 127.61, 127.42, 127.34, 127.17, 126.80, 126.49, 126.21, 126.01, 123.84, 99.77, 97.85, 84.53, 79.95, 79.77, 75.29, 74.75, 74.40, 73.88, 73.83, 73.19, 72.52, 71.20, 70.60, 69.20, 68.05, 66.90, 66.74, 60.30, 55.76, 20.80 (3Ac), 20.67 (Ac), 16.89 (CH_3); elemental analysis calcd (%) for $C_{72}H_{73}O_{19}NS$: C 67.12, H 5.71, N 1.09, S 2.49; found C 67.09, H 5.81, N 1.00, S 2.49.

Methyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-galactopyranoside (15): *p*-TsOH · H_2O (1.021 g) was added to a stirred solution of compound **14** (4.45 g, 18.94 mol) and α,α-dimethoxytoluene (4.32 g, 28.40 mmol) in dry acetonitrile (139 mL), and stirring was continued overnight at room temperature. Triethylamine was then added and the mixture concentrated under reduced pressure. The crude product was applied to a column of silica gel and eluted with dichloromethane/methanol 30:1 to give pure compound **15** (86 %) as a white solid. $R_f = 0.39$ (hexane/ethyl acetate 1:1); ^{1}H NMR (CD_3OD, 400 MHz): δ = 7.58 – 7.56 (m, 2H; ArH), 7.40 – 7.37 (m, 3H; ArH), 5.63 (s, 1H; benzylidene proton), 4.84 (d, $J = 3.6$ Hz, 1H; H-1), 4.42 (dd, J = 3.2, 11.2 Hz, 1H; H-2), 4.32 – 4.26 (m, 2H; H-3, H-6b), 4.11 (dd, $J = 1.2$, 12.8 Hz, 1H; H-6a), 3.88 (dd, $J = 3.2$, 10.8 Hz, 1H; H-5), 3.71 (s, 1H; H-4), 3.41 (s, 3H; OCH_3), 2.21 (s, 3H; Ac); ^{13}C NMR ($CDCl_3$,

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100.6 MHz): $\delta = 172.20$ (C=O), 137.60, 128.50, 128.00, 126.30, 100.50, 99.50, 75.50, 69.20, 67.50, 62.50, 55.00, 50.00, 21.00 (Ac); elemental analysis calcd (%) for $C_{16}H_{21}O_6N$: C 59.43, H 6.55, N 4.33; found C 59.24, H 6.45, N 4.15.

Methyl (2,3,4-tri-*O*-acetyl-6-*O*-pivaloyl-*β*-D-galactopyanosyl)-(1 → 3)-2-acetamido-4,6-*O*-benzylidene-2-deoxy-*α*-D-galactopyranoside (17): A mixture of compound **15** (3.0 g, 9.28 mmol) and powered $Hg(CN)_2$ (4.06 g) in benzene/nitromethane 1:1 (100 mL) was heated until 50 mL of solvent had been distilled off. The temperature was then adjusted to 40 to 45 °C, and 6-*O*-pivaloyl-2,3,4-tri-*O*-acetyl-*α*-D-galactopyranoside bromide **16** (8.0 g) was added and the stirring was continued for 12 h at the same temperature. The mixture was then diluted with benzene and washed with sat. aq. $NaHCO_3$, 10% KI, water, dried (Na_2SO_4) and concentrated. The crude residue was applied to a column of silica gel and eluted with dichloromethane/methanol 20:1 to give compound **17** (70%) as an amorphous solid. $R_f = 0.47$ (CH_2Cl_2/MeOH 20:1); ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 7.53 - 7.50$ (m, 2H; ArH), 7.50 – 7.00 (m, 3H; ArH), 5.60 – 5.50 (m, 2H; NHAc, benzylidene proton), 5.35 (d, $J = 2.8$ Hz, 1H; H^b-4), 5.18 (dd, 1H; H^b-2), 4.98 (dd, 1H; H^b-3), 4.88 (d, $J = 3.2$ Hz, 1H; H^a-1), 4.78 (d, $J = 7.6$ Hz, 1H; H^b-1), 4.72 – 4.60 (m, 1H; H^a-2), 4.31 – 4.02 (m, 5H; H^a-4, H^a-6a, H^b-6b, H^b-6a, H^a-6a), 4.00 – 3.85 (m, 2H; H^a-3, H^b-5), 3.60 (s, 1H; H^a-5), 3.40 (s, 3H; OCH_3), 2.20 (s, 3H; Ac), 2.05 (s, 3H; Ac), 2.00 (s, 3H; Ac), 1.98 (s, 3H; Ac), 1.27 (s, 9H; *t*Bu); ^{13}C NMR ($CDCl_3$, 100.6 MHz): $\delta = 178.00$ (C=O), 170.40 (C=O), 170.20 (C=O), 169.53 (C=O), 169.50 (C=O), 101.58, 100.59, 99.50, 76.00, 74.80, 71.00 (2C), 69.58, 69.00, 67.00, 63.00, 61.20, 55.60, 48.90, 38.90 [$C(CH_3)_3$], 27.00 ($3CH_3$), 23.50 (NAc), 20.90 (Ac), 20.80 (Ac), 20.50 (Ac); elemental analysis calcd (%) for $C_{33}H_{45}O_{15}N$: C 56.97, H 6.52, N 2.01; found C 56.51, H 6.35, N 1.73.

Methyl (2,3,4-tri-*O*-acetyl-6-*O*-pivaloyl-*β*-D-galactopyranosyl)-(1 → 3)-2-acetamido-2-deoxy-*α*-D-galactopyanoside (18): Compound **17** (1.2 g, 1.73 mmol) was taken up in 60% aqueous acetic acid and stirred for 1.5 h at 60 to 65 °C. The solution was then concentrated under reduced pressure, and the crude mixture applied to a short column of silica gel and eluted with dichloromethane/methanol 20:1 to give pure compound **18** (735 mg, 70%) as an amorphous solid. $R_f = 0.07$ (CH_2Cl_2/MeOH 20:1); ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 5.80$ (d, $J = 9.8$ Hz, 1H; NHAc), 5.38 (d, $J = 2.4$ Hz, 1H; H^b-4), 5.15 (dd, 1H; H^b-2), 5.02 (dd, $J = 3.6$, 10.6 Hz, 1H; H^b-3), 4.69 (d, $J = 4.0$ Hz, 1H; H^a-1), 4.67 (d, $J = 7.6$ Hz, 1H; H^b-1), 4.52 – 4.47 (m, 1H; H^a-2), 4.13 – 4.11 (m, 3H), 3.99 – 3.98 (m, 1H), 3.89 – 3.82 (m, 1H), 3.78 – 3.74 (m, 3H), 3.40 (s, 3H; OCH_3), 2.17 (s, 3H; Ac), 2.07 (s, 3H; Ac), 1.97 (s, 3H; Ac), 1.95 (s, 3H; Ac); ^{13}C NMR ($CDCl_3$, 100.6 MHz): $\delta = 178.06$ (C=O), 170.25 (C=O), 170.22 (C=O), 169.88 (C=O), 169.76 (C=O), 101.99, 99.06, 77.97, 71.11, 70.79, 69.78, 69.58, 68.89, 67.14, 62.90, 61.49, 55.28, 48.03, 39.00, 27.19 ($3CH_3$), 23.54 (NAc), 20.83 (Ac), 20.75 (Ac), 20.67 (Ac); elemental analysis calcd (%) for $C_{26}H_{41}O_{15}N$: C 51.39, H 6.80, N 2.31; found C 51.20, H 6.74, N 2.32.

Methyl (6-*O*-pivaloyl-*β*-D-galactopyanosyl)-(1 → 3)-2-acetamido-4,6-*O*-benzylidene-2-deoxy-*α*-D-galactopyranoside (19): 1M CH_3ONa/CH_3OH was added dropwise at −15 to −20 °C to a solution of compound **17** (3.5 g, 5.03 mmol) in dichloromethane/methanol (44 mL, 1:1), until the pH of solution was adjusted to ≈10. The mixture was stirred at the same temperature for 20 min, then quenched with acetic acid and concentrated under reduced pressure. The crude mixture so obtained was passed through a short column of silica gel and eluted with dichloromethane/methanol 20:1 to give pure compound **19** (2.31 g, 81%) as an amorphous solid. $R_f = 0.25$ (CH_2Cl_2/MeOH 20:1); ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 7.30 - 7.00$ (m, 2H; ArH), 7.00 – 6.50 (m, 3H; ArH), 5.95 (d, $J = 7.5$ Hz, 1H; NHAc), 5.60 – 5.50 (s, 1H; benzylidene proton), 4.85 (d, $J = 3.4$ Hz, 1H; H^a-1), 4.70 – 4.60 (m, 1H; H^a-2), 4.40 – 4.30 (m, 2H; H^a-4, H^a-6b), 4.30 – 4.20 (m, 2H; H^b-6b, H^a-6a), 4.15 (d, $J = 8.0$ Hz, 1H; H^b-1), 4.05 (dd, 1H; H^b-6a), 3.81 (dd, 1H; H^a-3), 3.69 (d, 1H; H^b-4), 3.62 – 3.58 (m, 2H; H^b-2, H^b-5), 3.53 (t, 1H; H^a-5), 3.40 (s, 3H; OCH_3), 3.33 (dd, 1H; H^b-3), 2.01 (s, 3H; Ac), 1.27 (s, 9H; *t*Bu); ^{13}C NMR ($CDCl_3$, 100.6 MHz): $\delta = 178.20$ (C=O), 171.80 (C=O), 138.00, 129.21, 128.10, 127.00, 105.80, 101.40, 99.60, 77.50, 76.00, 73.20, 72.50, 70.50, 69.20, 68.10, 63.10 (2C), 55.50, 48.50, 38.50, 27.50 ($3CH_3$), 23.50 (NAc); elemental analysis calcd (%) for $C_{27}H_{39}O_{12}N$: C 56.93, H 6.90, N 2.46; found C 56.87, H 6.82, N 2.30.

Phenyl (methyl *N*-acetyl-5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-2-*β*-thio-D-*glycero*-D-*galacto*-2-nonulopyranosid)onate (21): (±)-10-Camphorsulfonic acid (232 mg) was added to a solution of compound **20** (6.64 g, 11.38 mmol) in isopropenylacetate (58 mL). After 16 h at 65 °C, the mixture was quenched by adding triethylamine then concentrated under reduced pressure to a crude product residue, which was applied to a short column of silica gel and eluted with hexane/ethyl acetate 1:1 to give pure compound **21** in quantitative yield. $R_f = 0.53$ (hexane/ethyl acetate 1:1) $[\alpha]_D = -58.2$ ($c = 1.0$, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 7.51 - 7.49$ (m, 2H; ArH), 7.39 – 7.34 (m, 3H; ArH), 5.85 (ddd, 1H; H-4), 5.64 (dd, $J = 2.0$, 9.8 Hz, 1H; H-5), 5.25 (t, 1H; H-7), 4.90 (m, 1H; H-8), 4.31 (dd, $J = 1.6$, 12.2 Hz, 1H; H-9a), 4.14 – 4.03 (m, 2H; H-9b, H-6), 3.62 (s, 3H; $COOCH_3$), 2.74 (dd, $J_{3e,4} = 4.0$, 12.0 Hz, 1H; H-3e), 2.39 (s, 3H; Ac), 2.26 (s, 3H; Ac), 2.09 (dd, 1H; H-3a), 2.08 (s, 6H; 2Ac), 1.99 (s, 6H; 2Ac); ^{13}C NMR ($CDCl_3$, 100.6 MHz): $\delta = 175.06$ (C=O), 173.74 (C=O), 170.53 (C=O), 170.44 (C=O), 170.36 (C=O), 169.75 (C=O), 168.69 (C=O), 136.63, 130.08, 130.20, 129.46, 89.19, 72.28, 70.48, 69.04, 67.08, 62.44, 57.82, 52.85, 38.97 (CH_2), 28.15 (Ac), 25.97 (Ac), 21.02 (Ac), 20.99 (Ac), 20.87 (Ac); elemental analysis calcd (%) for $C_{28}H_{35}O_{13}NS$: C 53.75, H 5.64, N 2.24, S 5.13; found C 53.73, H 5.67, N 2.06, S 5.12.

Trisaccharide 22: A solution of compound **21** (1.61 g, 2.58 mmol), compound **19** (1.63 g, 2,35 mmol), *N*-iodosuccinimide (NIS, 1.83 g, 8.13 mmo) in dry dichloromethane/acetonitrile (60 mL, 1:1) containing 3 Å MS (12 g) was stirred at −45 to −40 °C for 2 h under N_2 atmosphere. Trifluoromethanesulfonic acid (TfOH) (237 μL) in dry acetonitrile (2 mL) was then added dropwise and stirring continued for 6 h. Additional portions of donor **21** (550 mg) and TfOH (70 μL) were then added and stirring was continued for a total of 12 h. The mixture was neutralized with sat. aq. sodium bicarbonate. Solids were filtered off and the organic layer washed with sat. aq. $NaHCO_3$, 10% $Na_2S_2O_3$, water, dried (Na_2SO_4), and concentrated under reduced pressure to a crude product. The product was applied to a column of silica gel and eluted with dichloromethane/methanol 30:1 to give a pure compound **22** (1.26 g, 45%) as a glassy white solid. $R_f = 0.15$ (CH_2Cl_2/MeOH 30:1); ^{1}H NMR ($CDCl_3$, 600 MHz): $\delta = 7.57 - 7.54$ (m, 2H; ArH), 7.37 – 7.33 (m, 3H; ArH), 6.38 (d, $J = 8.4$ Hz, 1H; NHAc), 5.56 – 5.47 (m, 3H; H^c-4, benzylidene proton, H^c-8), 5.14 (dd, $J = 8.8$, 8.4 Hz, 1H; H^c-7), 5.01 (d, $^3J_{1,2} = 3.6$ Hz, 1H; H^a-1), 4.95 (dd, $J = 10.0$ Hz, 1H; H^c-6), 4.70 – 4.69 (m, 1H; H^a-2), 4.42 (d, $^3J_{1,2} = 8.0$ Hz, 1H; H^b-1), 4.37 (d, $^3J_{3,4} = 2.8$ Hz, 1H; H^a-4), 4.34 – 4.24 (m, 4H; H^c-5, H^c-9b, H^b-6b, H^b-6a), 4.15 (dd, $J = 9.6$, 10.0 Hz, 1H; H^b-3), 4.04 (d, $J = 12.6$ Hz, 1H; H^a-6a), 3.95 – 3.88 (m, 2H; H^c-9a, H^a-3), 3.85 (s, 3H; $COOCH_3$), 3.71 – 3.65 (m, 2H; H^b-2, H^b-5), 3.61 (s, 1H; H^a-5), 3.56 (s, 1H; H^b-4), 3.42 (s, 3H; OCH_3), 2.87 (dd, $J = 4.8$, 13.0 Hz, 1H; H^c-3e), 2.37 (s, 3H; NAc), 2.31 (s, 3H; NAc), 2.16 (s, 3H; Ac), 2.15 (s, 3H; Ac), 2.06 (s, 3H; Ac), 2.03 (s, 3H; Ac), 2.02 (s, 3H; Ac), 1.65 (t, $J_{gem} = 12.6$ Hz, 1H; H^c-3a), 1.23 (t, 9H; *t*Bu); ^{13}C NMR ($CDCl_3$, 100.6 MHz): $\delta = 172.50$ (C=O), 171.98 (C=O), 171.80 (C=O), 171.00 (C=O), 170.30 (C=O), 169.80 (C=O), 168.2 (C=O), 137.91, 128.91, 128.10, 126.53, 105.61, 102.31, 99.81, 99.50, 76.10, 76.05, 72.12, 70.00, 69.40, 68.83, 68.00, 67.88, 67.00, 66.55, 63.20, 62.81, 62.00, 56.50, 55.21, 53.00, 49.55, 38.50, 27.20 ($3CH_3$), 23.50 (2NAc), 21.30 (2Ac), 20.81 (Ac), 20.56 (Ac), 20.51 (Ac); elemental analysis calcd (%) for $C_{49}H_{68}O_{25}N_2$: C 54.24, H 6.32, N 2.58; found C 53.98, H 6.14, N 2.27.

Trisaccharide 24: Acetic anhydride (5 mL) was added to a solution of compound **22** (641 mg, 0.53 mmol) and DMAP (10 mg) in dry pyridine (5 mL). The mixture was stirred overnight at room temperature then concentrated under reduced pressure, and the crude mixture was passed through a short column of silica gel eluted with dichloromethane/methanol 40:1 to give acetylated **23** (600 mg) which was directly used for the next step. Acetylated **23** (600 mg) in 60% aqueous HOAc was stirred for 2 – 3 h at 60 to 65 °C. The solution was concentrated and the crude product applied to a short column of silica gel and eluted with dichloromethane/methanol 25:1 to give compound **24** (428 mg, 76%) as a glassy white solid. $R_f = 0.21$ (CH_2Cl_2/MeOH 25:1); ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 6.25$ (d, $J = 8.8$ Hz, 1H; NHAc), 5.70 – 5.60 (m, 1H; H^c-8), 5.60 – 5.50 (m, 1H; H^c-4), 5.15 (dd, $J = 7.6$, 8.6 Hz, 1H; H^c-7), 5.10 – 5.00 (m, 2H; H^b-2, H^b-4), 4.83 (d, $J = 3.6$ Hz, 1H; H^a-1), 4.73 (d, $J = 7.6$ Hz, 1H; H^b-1), 4.65 – 4.50 (m, 3H; H^c-6, H^b-3, H^a-2), 4.40 – 4.20 (m, 2H; H^c-5, H^c-9b), 4.20 – 4.10 (m, 2H; H^a-4, H^b-6b), 4.00 – 3.90 (m, 2H; H^b-5, H^a-6b), 3.85 (s, 3H; $COOCH_3$), 3.85 – 3.70 (m, 5H; H^a-6a, H^a-5, H^b-6b, H^c-9a, H^a-3), 3.40 (s, 3H; OCH_3), 2.62 (dd, $J = 4.8$, 12.6 Hz, 1H; H^c-3e), 2.36 (s, 3H; Ac), 2.04 (s, 6H; 2Ac), 2.03 (s, 6H; 2Ac), 2.02 (s, 6H; 2Ac), 1.91 (s, 6H; 2Ac), 1.66 (t, $J_{gem} = 12.6$ Hz, 1H; H^c-3a), 1.27 (s, 9H; *t*Bu); ^{13}C NMR ($CDCl_3$, 100.6 MHz): $\delta = 177.58$ (C=O), 174.20 (C=O), 173.52 (C=O), 171.50 (C=O), 171.30 (C=O), 170.20 (C=O), 170.10 (C=O), 169.70 (C=O), 168.00 (C=O), 102.50, 98.92, 96.53, 78.20, 71.43, 70.79, 69.85, 69.45, 69.41, 69.21, 67.60, 67.40, 67.21, 67.03, 63.00, 61.02, 55.98, 55.10, 53.12, 48.10, 38.20, 27.31 ($3CH_3$), 23.12 (Ac), 21.53 (Ac), 21.21 (Ac),

20.59 (Ac), 20.55 (Ac); elemental analysis calcd (%) for $C_{46}H_{68}O_{27}N_2$: C 51.11, H 6.34, N 2.59; found C 50.84, H 6.23, N 2.44.

Glycosylation procedure B with NIS/TfOH promoter: A solution of acceptor (1 mmol), donor (1.05 – 1.1 mmol), NIS (3.0 mmol) in dry dichloromethane containing 4 Å MS (500 – 800 mg per mL) was stirred at -65 to $-60\,^{\circ}C$ for 2 h under N_2 atmosphere. TfOH (25 μL – 32 μL per mmol NIS) in dry dichloromethane (2 mL) was then added dropwise and stirring was continued at the same temperature for 1.5 – 2 h. Reaction monitored by TLC. The mixture was neutralized with sat. aq. sodium bicarbonate, solids were filtered off and the organic layer washed with sat. aq. $NaHCO_3$, 10% $Na_2S_2O_3$, water, dried (Na_2SO_4), and concentrated under reduced pressure. The resultant mixture was purified on a column of silica gel eluted with dichloromethane/methanol to give pure product.

Tetrasaccharide 25: Yield: 1.03 g, 89% as a glassy white solid from acceptor **18**. $R_f = 0.18$ (CH_2Cl_2/MeOH 30:1); ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 7.96 - 7.00$ (m, 11H; ArH), 5.81 (d, 1H, $J = 9.8$ Hz; NHAc), 5.80 – 5.75 (m, 2H), 5.38 (d, 1H), 5.28 (d, 1H), 5.15 (dd, 1H), 5.07 (dd, 1H), 5.02 (dd, 1H), 4.97 (d, $J_{gem} = 12.8$ Hz, 1H; $OCH_AC_{10}H_7$, ABq), 4.90 (dd, 1H), 4.75 (d, $J_{gem} = 11.2$ Hz, 1H; $OCH_BC_{10}H_7$, ABq), 4.69 (d, 1H), 4.59 (d, 1H), 4.67 (d, 1H), 4.52 – 4.47 (m, 1H), 4.37 (t, 1H), 4.13 – 4.11 (m, 3H), 4.09 – 4.03 (m, 3H), 3.99 – 3.98 (m, 1H), 3.89 – 3.82 (m, 3H), 3.76 – 3.78 (m, 3H), 3.74 (m, 1H), 3.63 (t, 1H), 3.40 (s, 3H; OCH_3), 2.17 (s, 3H; Ac), 2.11 (s, 3H; Ac), 2.10 (s, 3H; Ac), 2.08 (s, 3H; Ac), 2.07 (s, 3H; Ac), 1.98 (s, 3H; Ac), 1.97 (s, 3H; Ac), 1.95 (s, 3H; Ac),1.93 (s, 3H; Ac), 1.27 (s, 9H; *t*Bu); ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ =170.81 (C=O), 170.79 (C=O), 170.53 (C=O), 170.11 (C=O), 170.07 (C=O), 169.99 (C=O), 169.5 (C=O), 169.48 (C=O), 168.89 (C=O), 167.67 (C=O), 134.51, 128.18, 128.70, 127.95, 127.09, 126.61, 126.46, 126.40, 126.21, 126.10, 101.78, 100.41, 98.93, 98.46, 77.98, 77.80, 75.57, 74.64, 74.05, 71.22, 71.02, 70.92, 70.70, 70.50, 70.28, 69.24, 68.77, 67.56, 66.86 (2C), 61.15, 60.92, 55.15, 54.45, 47.85, 27.13 (3CH_3), 23.48 (Ac), 20.75 (3Ac), 20.67 (3Ac), 20.58 (2Ac); elemental analysis calcd (%) for $C_{67}H_{82}O_{31}N_2$: C 57.02, H 5.86, N 1.98; found C 56.97, H 5.83, N 1.73.

Tetrasaccharide 26: Compound **25** (450 mg, 0.32 mmol) in a mixture of ethanol (18 mL) and NH_2-$NH_2 \cdot H_2O$ (2 mL) was stirred at 80 to 85 °C for 2 h under N_2 atmosphere. The mixture was concentrated and dried for 1.5 h under reduced pressure then acetylated with pyridine/acetic anhydride (10 mL, 1:1) overnight at room temperature. Pyridine was removed under reduced pressure and the resultant product was passed through a silica gel column eluted with dichloromethane/methanol 10:1 to give compound **26** (360 mg, 83%) as an amorphous solid. $R_f = 0.3$ (CH_2Cl_2/MeOH 10:1); ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 7.96 - 7.30$ (m, 7H; ArH), 5.81 – 5.79 (m, 2H), 5.80 – 5.77 (m, 2H), 5.38 (d, 1H), 5.26 (d, 1H), 5.15 (dd, 1H), 5.07 (dd, 1H), 5.02 (dd, 1H), 4.97 (d, $J_{gem} = 12.6$ Hz, 1H; $OCH_AC_{10}H_7$, ABq), 4.88 (dd, 1H), 4.73 (d, $J_{gem} = 11.6$ Hz, 1H; $OCH_BC_{10}H_7$, ABq), 4.66 (d, 1H), 4.58 (d, 1H), 4.55 (d, 1H), 4.53 – 4.48 (m, 1H), 4.40 – 4.34 (m, 1H), 4.14 – 4.10 (m, 3H), 4.09 – 4.03 (m, 3H), 3.99 – 3.96 (m, 1H), 3.89 – 3.83 (m, 3H), 3.76 – 3.78 (m, 3H), 3.75 (m, 1H), 3.64 (m, 1H), 3.34 (s, 3H; OCH_3), 2.18 (s, 3H; Ac), 2.15 (s, 3H; Ac), 2.12 (s, 3H; Ac), 2.11 (s, 3H; Ac), 2.09 (s, 3H; Ac), 2.08 (s, 3H; Ac), 1.98 (s, 3H; Ac), 1.98 (s, 3H; Ac), 1.96 (s, 3H; Ac), 1.95 (s, 3H; Ac), 1.26 (s, 9H; *t*Bu); elemental analysis calcd (%) for $C_{63}H_{84}O_{31}N_2$: C 55.42, H 6.20, N 2.05; found C 55.12, H 6.16, N 1.78.

General procedure for removal of the 2-naphthylmethyl (NAP) group: DDQ (1.5 mmol) was added to a solution of compound **26** or **34** (1 mmol) in dichloromethane/methanol/H_2O 4:1:trace (10 mL). The mixture was stirred at room temperature for 12 – 20 h and the reaction monitored by TLC. The mixture was concentrated under reduced pressure to a crude residue then redissolved in dichloromethane (50 – 100 mL) and washed with sat. aq. $NaHCO_3$ (3 × 100 mL), water, dried (Na_2SO_4), and concentrated to a crude product, which was purified over a short column of silica gel eluted with dichloromethane/methanol to yield pure compound.

Tetrasaccharide 27: Yield: 242 mg, 75% as an amorphous solid from **26**. $R_f = 0.38$ (CH_2Cl_2/MeOH 15:1) $[\alpha]_D = 58.9$ ($c = 1.0$, $CHCl_3$); ^{1}H NMR (CD_3OD, 400 MHz): $\delta = 5.36 - 5.34$ (m, 2H; NHAc), 5.13 – 4.99 (m, 4H), 4.76 – 4.74 (m, 1H), 4.63 (d, $^3J_{1,2} = 3.6$ Hz, 1H; H^a-1), 4.52 (d, 1H, $J =$ 8.4 Hz), 4.40 (dd, 1H), 4.24 – 4.00 (m, 10H), 3.86 – 3.84 (m, 4H), 3.76 (dd, 1H), 3.52 (dd, 1H), 3.44 – 3.36 (m, 1H), 3.36 (s, 3H; OCH_3), 3.31 (s, 1H), 2.14 (s, 3H; Ac), 2.13 (s, 3H; Ac), 2.10 (s, 3H; Ac), 2.09 (s, 3H; Ac), 2.07 (s, 3H; Ac), 2.04 (s, 3H; Ac), 2.03 (s, 3H; Ac), 1.98 (s, 3H; Ac), 1.93 (s, 6H; 2Ac), 1.88 (s, 3H; Ac), 1.19 (s, 9H; *t*Bu); ^{13}C NMR ($CDCl_3$, 100.6 MHz): $\delta = 184.17$ (C=O), 178.17 (CO), 178.06 (C=O), 177.05 (C=O), 176.98 (C=O), 176.88 (C=O), 176.48 (C=O), 176.38 (C=O), 176.15 (C=O), 176.13 (C=O), 107.38 (2C), 106.79, 105.03, 81.65, 81.59, 79.82, 79.40, 77.42, 77.35, 77.18, 76.73, 76.67, 75.73, 75.19, 75.08, 74.81, 73.67, 73.39, 76.31, 67.10, 65.92, 60.61, 60.22, 55.40, 44.73, 27.85 (3CH_3), 27.80 (Ac), 27.65 (Ac), 26.00 (Ac), 25.80 (Ac), 25.60 (Ac), 25.42 (Ac), 25.40 (Ac); elemental analysis calcd (%) for $C_{52}H_{76}O_{31}N_2$: C 50.78, H 6.25, N 2.29; found C 51.0, H 6.23, N 2.01.

Tetrasaccharide 28: $SO_3 \cdot$pyridine complex (62 mg, 0.39 mmol) was added to a solution of compound **27** (320 mg, 0.26 mmol) in dry pyridine (6 mL). The mixture was stirred at 0 to 5 °C for 6 h. The reaction was quenched with methanol and the mixture concentrated under reduced pressure to a crude residue, which was applied to a short column of silica gel eluted with dichloromethane/methanol 20:1 to yield pure compound **28** (280 mg, 81%) as a glassy white solid. $R_f = 0.36$ (CH_2Cl_2/MeOH 10:1); ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 5.39 - 5.37$ (m, 2H; NHAc), 5.15 – 5.09 (m, 1H), 5.08 – 5.06 (m, 3H), 5.04 – 5.01 (m, 2H), 4.81 – 4.77 (m, 2H), 4.66 (d, $J = 3.2$ Hz, 1H), 4.55 (d, $J = 8.8$ Hz, 1H), 4.43 (dd, $J = 3.2$, 10.4 Hz, 1H), 4.22 (dd, 1H), 4.17 – 4.05 (m, 7H), 3.93 – 3.87 (m, 5H), 3.79 (dd, 1H), 3.53 (dd, 1H), 3.43 – 3.41 (m, 1H), 3.38 (s, 3H; OCH_3), 3.33 (s, 1H), 2.16 (s, 3H; Ac), 2.15 (s, 3H; Ac), 2.12 (s, 3H; Ac), 2.09 (s, 3H; Ac), 2.07 (s, 3H; Ac), 2.04 (s,3H; Ac), 2.00 (s,3H; Ac), 1.97 (s, 6H; 2Ac), 1.91 (s, 3H; Ac), 1.27 (s, 9H; *t*Bu); ^{13}C NMR ($CDCl_3$, 100.6 MHz): $\delta = 184.32$ (C=O), 178.39 (C=O), 178.27 (C=O), 177.24 (C=O), 177.21 (C=O), 177.09 (C=O), 177.07 (C=O), 176.68 (C=O), 176.62 (C=O), 176.39 (C=O), 108.05, 107.54, 106.58, 105.07, 81.62, 80.18, 79.73, 79.63, 77.74, 77.46, 76.88, 76.84, 76.57, 76.14, 75.71, 75.35, 75.10, 73.90, 73.53, 71.73, 67.46, 66.99, 60.89, 60.35, 55.60, 32.59, 28.00, 27.96 (Ac), 26.18 (Ac), 25.95 (Ac), 25.58 (Ac), 25.19 (Ac).

Deprotected tetrasaccharide 1: A catalytic amount of 1M sodium methoxide (100 μL) was added to a solution of compound **28** (120 mg, 0.092 mmol) in aqueous methanol (6 mL). The mixture was stirred at room temperature for 24 h then neutralized with acetic acid and concentrated under reduced pressure. The product was dissolved in water and treated with Amberlite IR 120 (Na^+) cation exchange resin, filtered, and concentrated under reduced pressure to a crude mixture, which was applied to a short column of silica gel eluted with nC_3H_7OH/HOAc/H_2O 3:1:1 to give a pure compound **1** (30 mg, 38%) as a glassy white solid. $R_f =$ 0.1 (nC_3H_7OH/HOAc/H_2O 3:1:1); ^{1}H NMR (CD_3OD+D_2O, 600 MHz): $\delta = 4.76$ (d, $J = 3.2$ Hz, 1H; H^a-1), 4.59 (d, $J = 8.4$ Hz, 1H; H^c-1), 4.54 (d, $J = 7.8$ Hz, 1H; H^d-1), 4.46 (d, $J = 7.8$ Hz, 1H; H^b-1), 4.41 (dd, $J = 9.6$ Hz, 1H; H^c-6b), 4.34 – 4.30 (m, 2H; H^c-6a, H^a-2), 4.21 (d, $J = 3.0$ Hz, 1H; H^a-4), 4.12 (dd, 1H; H^a-5), 4.04 – 4.02 (m, 1H; H^a-6b), 4.01 – 3.99 (m, 1H; H^a-3), 3.94 (d, $J = 3.6$ Hz, 1H; H^d-4), 3.92 (d, $J = 3.0$ Hz, 1H; H^b-4), 3.80 – 3.72 (m, 9H; H^c-5, H^c-4, H^a-6a, H^b-6b, H^c-2, H^d-6b, H^b-6a, H^c-3, H^d-6a), 3.69 – 3.61 (m, 4H; H^d-5, H^d-3, H^b-5, H^b-3), 3.56 – 3.50 (m, 2H; H^d-2, H^b-2), 3.37 (s, 3H; OCH_3), 2.02 (s, 3H; Ac), 1.91 (s, 3H; Ac); ^{13}C NMR (D_2O+CD_3OD, 100.6 MHz): $\delta = 175.17$ (C=O), 174.99 (C=O), 105.31, 103.20, 102.51, 98.78, 78.30, 77.92, 76.01, 75.59, 73.22, 72.99, 71.68, 71.31, 71.07, 70.06, 69.95, 69.59, 69.29, 68.99, 66.97, 61.68, 61.58, 55.76, 55.58, 55.48, 49.20, 22.69 (Ac), 22.62 (Ac); FABMS (*m*/*z*) (positive ion mode) $C_{29}H_{49}O_{24}N_2SNa_2$: 887.3; found 887.4 $[M + Na]^+$.

Sialylated tetrasaccharide 29: See glycosylation procedure B, yield: 291 mg, 59% as an amorphous solid from acceptor **24**. $R_f = 0.32$ (CH_2Cl_2/MeOH 30:1); ^{1}H NMR ($CDCl_3$, 600 MHz): $\delta = 7.88 - 7.82$ (m, 6H; ArH), 7.80 – 7.62 (m, 2H; ArH), 7.58 – 7.42 (m, 3H; ArH), 6.08 (d, $J = 8.8$ Hz, 1H; NHAc), 5.74 (t, $J = 10.4$, 9.2 Hz, 1H; H^d-3), 5.62 (ddd, 1H; H^c-8), 5.55 (ddd, 1H; H^c-4), 5.36 (d, $J_{1,2} = 8.8$ Hz, 1H; H^d-1), 5.21 (d, $J = 2.8$ Hz, 1H; H^e-4), 5.12 (dd, $J = 2.4$, 9.8 Hz, 1H; H^c-7), 5.02 – 4.98 (m, 3H; H^b-2, H^e-2, $OCHC_{10}H_7$, ABq), 4.80 (dd, $J = 3.6$, 10.6 Hz, 1H; H^e-3), 4.69 – 4.66 (m, 2H; $OCHC_{10}H_7$, ABq, H^b-1), 4.62 – 4.55 (m, 2H; H^c-6, H^b-3), 4.51 (d, $J_{1,2} = 8.0$ Hz, 1H; H^e-1), 4.35 – 4.23 (m, 5H; H^a-1, H^a-2, H^c-5, H^c-9b, H^d-2), 4.10 – 3.94 (m, 7H; H^d-4, H^e-6b, H^b-6b, H^d-6b, H^d-6a, H^e-6a, H^a-4), 3.88 – 3.80 (m, 7H; $COOCH_3$, H^b-4, H^a-6b, H^a-6a, H^c-9b), 3.77 – 3.64 (m, 5H; H^b-6a, H^a-5, H^b-5, H^d-5, H^a-3), 3.36 – 3.48 (m, 1H; H^e-5), 2.85 (s, 3H; OCH_3), 2.66 (dd, $J = 4.8$, 12.6 Hz, 1H; H^c-3e), 2.36 (s, 3H; Ac), 2.30 (s, 3H; Ac), 2.23 (s, 3H; Ac), 2.18 (s, 3H; Ac), 2.09 (s, 3H; Ac), 2.08 (s, 3H; Ac), 2.05 (s, 3H; Ac), 2.03 (s, 3H; Ac), 1.96 (s, 3H; Ac), 1.95 (s, 3H; Ac), 1.85 (s, 3H; Ac), 1.81 (s, 3H; Ac), 1.58 (t, $J = 12.4$ Hz, 1H; H^c-3a), 1.20 (s, 9H; *t*Bu); ^{13}C NMR ($CDCl_3$, 100.6 MHz): $\delta = 177.79$ (C=O), 174.19 (C=O), 173.78 (C=O), 171.48 (C=O), 171.23 (C=O), 170.47 (C=O), 170.37 (C=O), 170.27 (C=O), 170.19 (C=O), 170.12 (C=O), 170.09 (3C=O), 169.78 (C=O), 169.76 (C=O), 168.96 (C=O), 168.08 (C=O), 167.81 (C=O), 135.38, 134.45, 134.15, 133.37, 133.33, 128.80, 128.09, 128.04, 127.22, 126.53, 126.31, 126.27, 126.25, 102.66, 100.51, 99.09, 98.40,

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96.78, 78.44, 75.69, 74.72, 74.19, 71.59, 71.32, 71.11, 70.77, 70.71, 70.59, 69.52, 69.42, 69.35, 69.18, 68.87, 67.68, 67.54, 67.49, 67.14, 67.01, 63.16, 61.04, 60.97, 56.00, 55.27, 54.44, 53.15, 48.32, 38.47, 27.27, 23.33 (Ac), 21.62 (Ac), 21.21 (Ac), 21.10 (Ac), 20.89 (Ac), 20.85 (Ac), 20.79 (Ac), 20.67 (Ac), 20.64 (Ac); elemental analysis calcd (%) for $C_{87}H_{111}O_{43}N_3$: C 55.38, H 5.93, N 2.23; found C 53.61, H 5.52, N 1.83.

Pentasaccharide 32: A solution of compound **29** (201 mg, 0.10 mmol), DMAP(5 mg), and acetic anhydride (3 mL) in dry pyridine (3 mL) was stirred at room temperature overnight. The reaction mixture was concentrated under reduced pressure to a crude residue which was applied to a short column of silica gel eluted with dichloromethane/methanol 25:1 to give a pure compound **30** (205 mg) in quantitative yield. A solution of compound **30** (218 mg, 0.106 mmol) in dichloromethane/methanol (5 mL, 4:1) was treated with DDQ (36 mg, 1.62 mmol) and th reaction mixture was stirred at room temperature for 16 h. An additional portion of DDQ (20 mg) was added and stirring continued for a total of 18 h. The reaction mixture was concentrated under reduced pressure, redissolved in dichloromethane (50 mL), then washed with sat. aq. $NaHCO_3$ (3 × 100 mL), water, dried (Na_2SO_4), and concentrated under reduced pressure to a crude product which was directly used for the next reaction. To a solution of the above product (196 mg) in dry pyridine (3 mL) was added SO_3·pyridine (25 mg) and the mixture was stirred at 0 to 5 °C for 6 h under N_2 atmosphere. The reaction mixture was quenched with methanol and concentrated under reduced pressure to a crude product which was treated in methanol (5 mL) with Amberlite IR 120 (Na^+) cation exchange resin at room temperature for 4 h. The mixture was filtered then concentrated to a crude product, which was applied to a short column of silica gel eluted with dichloromethane/methanol 15:1 to give **32** (135 mg, 63 %) as an amorphous solid. $R_f = 0.35$ (CH_2Cl_2/MeOH 15:1); ^{1}H NMR ($CDCl_3$, 600 MHz): δ = 7.68 – 7.45 (m, 4H; ArH), 5.68 (dd, J = 8.6, 10.0 Hz, 1H; H^d-3), 5.57 (ddd, 1H; H^c-4), 5.50 (ddd, 1H; H^c-8), 5.44 – 5.36 (m, 2H, $J_{1,2}$ = 7.6 Hz; H^d-1, J = 2.8 Hz; H^e-4), 5.30 (d, J = 3.2 Hz, 1H; H^a-4), 5.20 – 5.09 (m, 2H; H^c-7, H^e-3), 5.08 – 5.00 (m, 2H; H^b-4, H^e-2), 4.97 (d, $J_{1,2}$ = 8.0 Hz, 1H; H^e-1), 4.80 – 4.76 (m, 1H; H^b-2), 4.70 (d, $J_{1,2}$ = 8.0 Hz, 1H; H^b-1), 4.64 – 4.56 (m, 2H; H^b-3, H^c-6), 4.40 – 4.32 (m, 3H; H^d-6b, H^d-6a, H^c-5), 4.32 – 4.21 (m, 2H; H^a-2, H^c-9b), 4.18 (d, $J_{1,2}$ = 3.6 Hz, 1H; H^a-1), 4.16 – 4.05 (m, 6H; H^e-6b, H^c-6a, H^e-5, H^b-6b, H^a-6b, H^d-2), 4.04 – 3.92 (m, 4H; H^d-4, H^a-6a, H^c-9a, H^a-3), 3.92 – 3.84 (m, 5H; H^b-6a, $COOCH_3$, H^a-5), 3.84 – 3.80 (m, 1H; H^d-5), 3.42 (t, 1H; H^b-5), 3.00 – 2.96 (s, 3H; OCH_3), 2.60 (dd, J = 4.8, 12.6 Hz, 1H; H^c-3e), 2.36 (s, 3H; Ac), 2.34 (s, 3H; Ac), 2.24 (s, 3H; Ac), 2.16 (s, 3H; Ac), 2.14 (s, 3H; Ac), 2.10 (s, 3H; Ac), 2.08 (s, 3H; Ac), 2.07 (s, 3H; Ac), 2.06 (s, 3H; Ac), 2.04 (s, 3H; Ac), 2.02 (s, 3H; Ac), 1.98 (s, 3H; Ac), 1.94 (s, 3H; Ac), 1.92 (s, 3H; Ac), 1.88 (s, 3H; Ac), 1.52 – 1.44 (t, J = 12.6 Hz, 1H; H^c-3a), 1.20 (s, 9H; tBu); ^{13}C NMR (CD_3OD, 100.6 MHz): δ = 175.98 (C=O), 173.10 (C=O), 172.81 (C=O), 172.72 (C=O), 172.25 (C=O), 172.17 (C=O), 172.14 (C=O), 172.11 (C=O), 172.03 (C=O), 171.92 (C=O), 171.79 (C=O), 171.74 (C=O), 171.49 (C=O), 169.51 (C=O), 134.50, 124.57, 102.77, 101.52, 100.45, 99.99, 98.16, 76.58, 75.32, 74.94, 72.99, 72.81, 72.56, 71.93, 71.77, 71.57, 71.54, 71.50, 70.92, 70.76, 69.91, 68.99, 68.70, 68.41, 68.17, 66.41, 63.54, 62.49, 61.76, 57.19, 56.26, 55.52, 53.74, 50.52, 39.72 (CH_2), 27.69 (3CH_3), 23.17 (Ac), 21.85 (Ac), 21.82 (Ac), 21.32 (Ac), 20.98 (Ac), 20.90 (Ac), 20.87 (Ac), 20.74 (Ac), 20.63 (Ac), 20.59 (Ac).

Deprotected pentasaccharide 2: Lithium iodide (LiI, 400 mg, 3.0 mmol) was added to a solution of compound **32** (126 mg, 63 μmol) in dry pyridine (4 mL). The mixture was refluxed at 120 to 25 °C for 6 h under N_2 atmosphere. The dark yellow solution was then concentrated to dryness and co-evaporated with toluene to a corresponding carboxylic acid as a dark yellow amorphous solid which was directly used for the next reaction. A solution of the above in methanol (15 mL), was treated with NH_2-NH_2·H_2O (3 mL) solution for 4 h at 80 to 85 °C, the mixture was concentrated under reduced pressure, co-evaporated with toluene then acetylated with acetic anhydride/pyridine 1:1 in the presence of catalytic amount of DMAP at room temperature overnight. The acetylated mixture was concentrated and passed through a short column of silica gel eluted with dichloromethane/methanol 10:1 to give a bright yellow film. To a solution of this bright yellow film in methanol/water (2 mL, 1:1), was added a catalytic amount of 1M sodium methoxide (150 μL). The mixture was stirred at room temperature for 48 h and concentrated under reduced pressure to a crude mixture, which was then applied to a short column of silica gel eluted with $n$$C_3H_7OH$/HOAc/$H_2O$ 1:1:1 to give a pure compound **2** (26 mg, 36 %). R_f = 0.39 ($n$$C_3H_7OH$/HOAc/$H_2O$ 1:1:1); ^{1}H NMR (D_2O, 600 MHz): δ = 4.76 (d, $J_{1,2}$ = 3.2 Hz, 1H; H^a-1), 4.58 (d, $J_{1,2}$ = 8.0 Hz, 1H; H^d-1), 4.54 (d, $J_{1,2}$ = 7.6 Hz, 1H; H^e-1), 4.52 (d, $J_{1,2}$ = 8.0 Hz, 1H; H^b-1), 4.41 (dd, J = 10.0 Hz, 1H; H^d-6b), 4.35 – 4.28 (m, 2H; H^d-6a, H^a-2), 4.20 (d, $J_{3,4}$ = 3.6 Hz, 1H; H^a-4), 4.10 – 3.99 (m, 4H; H^a-5, H^b-3, H^a-6a, H^a-3), 3.90 – 3.56 (m, 20H; H^c-9b, H^c-5, H^b-6b, H^d-5, H^a-6a, H^d-2, H^e-5, H^c-7, H^c-4, H^e-3, H^b-5, H^b-6a, H^e-4, H^c-6, H^c-9a), 3.55 – 3.50 (m, 2H; H^e-2, H^b-2), 3.37 (s, 3H; OCH_3), 2.75 (dd, J = 4.4, 12.0 Hz, 1H; H^c-3e), 2.03 (s, 3H; Ac), 2.01 (s, 3H; Ac), 1.91 (s, 3H; Ac), 1.78 (t, J = 12.8 Hz, 11.6 Hz, 1H; H^c-3a); ^{13}C NMR (D_2O, 100.6 MHz): δ = 173.81 (C=O), 173.44 (C=O), 173.30 (C=O), 172.80 (C=O), 103.36, 101.33, 100.75, 98.52, 96.97, 76.17, 74.47, 74.19, 73.56, 71.62, 71.38, 71.32, 71.16, 70.65, 69.84, 69.58, 68.24, 67.88, 67.71, 67.47, 67.26, 66.90, 66.20, 65.09, 61.30, 59.91, 59.76, 53.95, 53.75, 50.50, 47.37, 38.54 (CH_2), 21.04 (Ac), 20.92 (Ac), 20.89 (Ac); FABMS (m/z) (positive ion mode) calcd for $C_{40}H_{66}O_{32}N_3SNa_2$: 1178.6; found 887.4 $[M+Na-Neu5Ac]^+$.

Hexasaccharide 33: See glycosylation procedure B, yield: 386 mg, 79 % as an amorphous solid from acceptor **24**. $R_f = 0.46$ (CH_2Cl_2/MeOH 30:1); ^{1}H NMR ($CDCl_3$, 600 MHz): δ = 7.96 – 7.89 (m, 4H; ArH), 7.70 – 7.69 (m, 2H; ArH), 7.56 – 7.51 (m, 4H; ArH), 7.29 – 7.01 (m, 16H; ArH), 6.09 (d, J = 9.6 Hz, 1H; NHAc), 5.65 (ddd, 1H; H^c-8), 5.57 (ddd, 1H; H^c-4), 5.13 – 5.11 (m, 3H; H^d-1, H^c-7, H^e-4), 5.03 – 4.98 (m, 3H; H^b-2, H^e-2, OCHAr, ABq), 4.85 – 4.73 (m, 4H; H^a-4, $J_{3,4}$ = 3.6 Hz, OCHAr, ABq, H^e-3, H^e-1, $J_{1,2}$ = 8.0 Hz), 4.72 – 4.70 (m, 2H, $J_{1,2}$ = 5.2 Hz; H^a-1, H^b-1), 4.69 – 4.56 (m, 3H; OCHAr, J_{gem} = 12.0 Hz, H^c-6, OCHAr, J_{gem} = 11.6 Hz, ABq), 4.45 (d, J_{gem} = 12.8 Hz, 1H; OCHAr, ABq), 4.37 – 4.19 (m, 7H; H^f-1, H^f-4, H^c-5, H^c-9b, H^a-2, H^d-4), 4.11 – 3.80 (m, 14H; H^e-6b, H^b-6b, H^a-6b, H^f-3, H^d-6b, H^b-6a, H^e-6a, $COOCH_3$, H^d-6a, H^c-9b, H^b-5, H^a-3), 3.73 – 3.56 (m, 2H; H^a-6a, H^f-2), 3.35 (t, J = 6.8, 7.2 Hz, 1H; H^e-5), 2.84 (s, 3H; OCH_3), 2.67 (dd, J = 5.2, 12.8 Hz, 1H; H^c-3e), 2.37 (s, 3H; Ac), 2.31 (s, 3H; Ac), 2.24 (s, 3H; Ac), 2.19 (s, 3H; Ac), 2.09 (s, 3H; Ac), 2.05 (s, 3H; Ac), 2.04 (s, 3H; Ac), 1.96 (s, 3H; Ac), 1.95 (s, 3H; Ac), 1.93 (s, 3H; Ac), 1.92 (s, 3H; Ac), 1.91 (s, 3H; Ac), 1.79 (s, 3H; Ac), 1.59 (t, J = 12.4 Hz, 1H; H^c-3a), 1.20 (s, 12H; tBu, CH_3-f); ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ = 177.70 (C=O), 174.10 (C=O), 173.72 (C=O), 173.07 (C=O), 171.37 (C=O), 171.15 (C=O), 170.35 (C=O), 170.19 (C=O), 170.05 (C=O), 170.02 (C=O), 169.89 (C=O), 169.75 (C=O), 169.73 (C=O), 169.68 (C=O), 168.80 (C=O), 167.98 (C=O), 139.10, 138.91, 138.51, 135.40, 134.14, 133.22, 128.71, 128.32, 128.27, 128.20, 128.08, 127.98, 127.50, 127.27, 127.21, 127.09, 126.83, 126.44, 126.12, 125.99, 102.60, 99.70, 99.26, 98.22, 97.46, 96.71, 79.90, 78.47, 75.38, 75.26, 74.58, 74.29, 73.91, 72.85, 72.44, 72.35, 71.50, 71.45, 71.16, 70.84, 70.67, 70.41, 69.44, 69.27, 69.23, 69.10, 68.71, 68.08, 67.49, 67.43, 67.33, 67.07, 68.82, 66.51, 63.13, 60.88, 60.18, 56.64, 55.92, 54.39, 53.08, 48.24, 38.38, 27.20 (3CH_3), 23.25 (Ac), 21.54 (Ac), 21.14 (Ac), 21.03 (Ac), 20.80 (Ac), 20.69 (Ac), 20.61 (Ac), 16.81 (CH_3); elemental analysis calcd (%) for $C_{109}H_{135}O_{46}N_3$: C 58.88, H 6.12, N 1.89; found C 58.73, H 5.88, N 1.59.

Hexasaccharide 34: A solution of compound **33** (284 mg, 0.12 mmol), DMAP(8 mg), dry pyridine (5 mL), and acetic anhydride(5 mL) was stirred at room temperature overnight. The reaction mixture was concentrated under reduced pressure to a crude product, which was passed through a short column of silica gel eluted with dichloromethane/methanol 25:1 to give a pure compound **34** (264 mg, 85 %) as an amorphous solid. $R_f = 0.59$ (CH_2Cl_2/MeOH 25:1); ^{1}H NMR ($CDCl_3$, 600 MHz): δ = 7.95 – 7.80 (m, 4H; ArH), 7.70 – 7.61 (m, 3H; ArH), 7.56 – 7.50 (m, 3H; ArH), 7.29 – 7.03 (m, 15H; ArH), 6.11 (d, J = 9.5 Hz, 1H; NHAc), 5.64 (ddd, 1H; H^c-8), 5.56 (ddd, 1H; H^c-4), 5.13 – 5.10 (m, 3H; H^d-1, H^c-7, H^e-4), 5.03 – 4.96 (m, 3H; H^b-2, H^e-2, OCHAr, ABq), 4.88 – 4.73 (m, 4H; H^a-4, $J_{3,4}$ = 3.6 Hz, OCHAr, ABq, H^e-3, H^e-1, $J_{1,2}$ = 8.1 Hz), 4.71 – 4.69 (m, 2H; $J_{1,2}$ = 5.2 Hz, H^a-1, H^b-1), 4.69 – 4.51 (m, 3H; OCHAr, J_{gem} = 12.4 Hz, ABq, H^c-6, OCHAr, J_{gem} = 12.6 Hz, ABq), 4.44 (d, J_{gem} = 12.8 Hz, 1H; OCHAr, ABq), 4.38 – 4.20 (m, 7H; H^f-1, H^f-4, H^c-5, H^c-9b, H^a-2, H^d-4), 4.12 – 3.81 (m, 14H; H^e-6b, H^b-6b, H^a-6b, H^f-3, H^d-6b, H^b-6a, H^e-6a, $COOCH_3$, H^d-6a, H^c-9b, H^b-5, H^a-3), 3.74 – 3.56 (m, 2H; H^a-6a, H^f-2), 3.36 (t, J = 6.9, 7.3 Hz, 1H; H^e-5), 2.85 (s, 3H; OCH_3), 2.67 (dd, J = 5.2, 12.8 Hz, 1H; H^c-3e), 2.38 (s, 3H; Ac), 2.33 (s, 3H; Ac), 2.26 (s, 3H; Ac), 2.19 (s, 3H; Ac), 2.09 (s, 3H; Ac), 2.06 (s, 3H; Ac), 2.04 (s, 3H; Ac), 1.98 (s, 3H; Ac), 1.96 (s, 3H; Ac), 1.94 (s, 3H; Ac), 1.93 (s, 6H; 2Ac), 1.91 (s, 3H; Ac), 1.80 (s, 3H; Ac), 1.59 (t, J = 12.6 Hz, 1H; H^c-3a), 1.21 (s, 12H; tBu, CH^f_3); ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ = 170.81 (C=O), 170.70 (C=O), 170.51 (C=O), 170.34 (C=O), 170.21 (C=O), 169.89 (C=O), 169.85 (C=O), 169.84 (C=O), 169.80 (C=O), 169.69 (C=O), 169.64 (C=O), 168.88 (C=O), 168.41 (C=O), 168.21 (C=O), 139.20, 138.26, 135.50, 134.50, 128.95, 128.50, 128.48, 128.46, 128.45, 128.43, 128.05, 127.52, 127.50, 127.45, 127.00, 126.57, 126.51, 126.41, 101.98, 99.75, 99.51, 98.40, 97.53, 96.58,

80.12, 75.25, 75.21, 74.65, 74.63, 74.02, 74.00, 73.21, 72.50, 71.98, 71.31, 70.45, 70.41, 70.35, 70.21, 69.81, 69.75, 69.10, 68.95, 68.00, 67.51, 67.20, 66.81, 66.40, 62.90, 60.85, 60.30, 56.50, 56.00, 54.98, 53.20, 53.19, 49.50, 38.50, 27.50, 23.10, 21.51, 21.30, 21.29, 21.29, 20.98, 20.95, 20.57, 20.56, 16.89 (CH_3); elemental analysis calcd (%) for $C_{111}H_{137}O_{47}N_3$: C 58.85, H 6.10, N 1.86; found C 58.80, H 6.00, N 1.83.

Hexasaccharide 35: See general procedure, yield: 262 mg, 80% as an amorphous solid from **34**. $R_f = 0.31$ (CH_2Cl_2/MeOH 30:1); ^{1}H NMR ($CDCl_3$, 600 MHz): $\delta = 7.26-7.17$ (m, 15H; ArH), 7.15–7.12 (m, 2H; ArH), 7.01–6.99 (m, 2H; ArH), 5.99 (d, $J = 8.4$ Hz, 1H; NHAc), 5.56–5.55 (m, 3H; H^c-4, H^c-7), 5.35 (d, $J = 2.8$ Hz, 1H), 5.27 (d, $J = 3.2$ Hz, 1H), 5.14–5.07 (m, 3H), 5.02 (dd, $J = 2.4$, 10.6 Hz, 1H), 4.98 (d, $J = 3.2$ Hz, 1H), 4.90–4.86 (m, 2H), 4.81 (d, $J_{gem} = 12.0$ Hz, 1H; OCHPh, ABq), 4.72–4.53 (m, 4H; H^a-1, 3OCHPh, ABq), 4.52 (dd, $J = 7.6$, 3.2 Hz, 1H), 4.38 (d, $J_{gem} = 12.4$ Hz, 1H; OCHPh), 4.36–3.36 (m, 6H; H^a-2, OCHPh, ABq, $COOCH_3$, H^a-3), 3.63 (s, 1H), 3.44–3.42 (m, 1H), 3.25 (t, 1H), 2.95 (s, 3H; OCH_3), 2.64 (dd, $J = 4.8$, 12.4 Hz, 1H; H^c-3e), 2.35 (s, 3H; Ac), 2.29 (s, 3H; Ac), 2.21 (s, 3H; Ac), 2.03 (s, 3H; Ac), 1.99 (s, 3H; Ac), 1.95 (s, 3H; Ac), 1.94 (s, 3H; Ac), 1.88 (s, 3H; Ac), 1.59–1.54 (m, 4H; Ac, H^c-3a), 1.21–1.17 (m, 12H; CH^f_3, *t*Bu); ^{13}C NMR ($CDCl_3$, 100.6 MHz): $\delta = 177.69$ (C=O), 174.13 (C=O), 173.8 (C=O), 171.75 (C=O), 171.50 (C=O), 171.45 (C=O), 171.04 (C=O), 170.63 (C=O), 170.45 (C=O), 170.21 (C=O), 170.06 (C=O), 169.98 (C=O), 169.86 (C=O), 169.73 (C=O), 168.01 (C=O), 134.18, 128.35, 128.27, 128.22, 128.11, 127.92, 127.55, 127.29, 127.26, 127.13, 101.69, 101.26, 98.67, 98.37, 97.82, 96.71, 79.92, 75.51, 75.03, 74.80, 74.37, 74.05, 72.98, 72.79, 72.48, 71.66, 71.15, 7076, 70.43, 70.14, 69.67, 69.35, 68.54, 67.38, 67.22, 67.13, 67.08, 66.68, 62.82, 60.54, 60.48, 60.44, 56.46, 56.01, 54.86, 53.06, 49.15, 38.46 (CH_2), 27.18 (3CH_3), 23.31 (Ac), 21.52 (Ac), 21.16 (Ac), 21.05 (Ac), 20.86 (Ac), 20.84 (Ac), 20.68 (Ac), 20.64 (Ac), 16.87 (CH_3); elemental analysis calcd (%) for $C_{101}H_{131}O_{47}N$: C 56.71, H 6.17, N 1.96; found C 56.78, H 5.65, N 1.92.

Hexasaccharide 36a: A solution of compound **35** (253 mg, 0.11 mmol), dry pyridine (5 mL), and SO_3·pyridine complex (26 mg, 0.39 mmol) was stirred at 0 to 5°C for 6 h. The mixture was quenched with methanol and concentrated under reduced pressure to a crude mixture, which was applied to a short column of silica gel eluted with dichloromethane/methanol 20:1 to give a pure compound **36** (156 mg, 81%) as a glassy white solid. A solution of compound **36** (120 mg, 50.2 μmol) and 10% Pd/C (300 mg) in dry dichloromethane/methanol (10 mL, 4:1) was stirred at room temperature for 6 h under hydrogen atmosphere. Solids were filtered off and the organic layer was concentrated to a crude residue, which was passed through a short column of silica gel eluted with dichloromethane/methanol 10:1 to give compound **36a** (138 mg) in quantitative yield. $R_f = 0.17$ (CH_2Cl_2/MeOH 10:1); ^{1}H NMR ($CDCl_3$, 600 MHz): $\delta = 8.00-7.80$ (m, 4H; ArH), 5.59–5.58 (m, 1H; H^c-4), 5.53–5.49 (m, 1H; H^c-8), 5.44 (d, 1H; H^e-4), 5.27 (d, $J = 2.8$ Hz, 1H; H^a-4), 5.19–5.16 (m, 2H, H^d-1, $J_{1,2} = 8.6$ Hz, H^c-7), 5.06 (d, $J = 3.2$ Hz, 1H; H^b-4), 4.81–4.76 (m, 2H; H^f-5, H^b-2), 4.71 (d, $J_{1,2} = 8.0$ Hz, 1H; H^b-1), 4.63–4.60 (m, 2H; H^c-6, H^b-3), 4.56 (d, $J_{1,2} = 4.0$ Hz, 1H; H^f-1), 4.52 (t, $J = 9.2$, 10.8 Hz, 1H; H^d-3), 4.44 (dd, $J = 2.0$, 10.8 Hz, 1H; H^d-6b), 4.38–4.32 (m, 2H; H^d-6a, H^c-5), 4.27–4.09 (m, 6H; H^a-2, H^c-9b, H^a-6b, H^d-2, H^e-1, H^a-3), 4.04–3.91 (m, 3H; H^d-4, H^a-6a, H^c-9a), 3.91–3.85 (m, 5H; H^a-5, $COOCH_3$), 3.81 (dd, $J = 3.6$, 10.2 Hz, 1H; H^f-3), 3.76 (dd, 1H; H^d-5), 3.70 (d, 1H; H^f-4), 3.37–3.33 (m, 3H), 2.99 (s, 3H; OCH_3), 2.59 (dd, $J = 5.2$, 12.4 Hz, 1H; H^c-3e), 2.37 (s, 3H; NAc), 2.35 (s, 3H; NAc), 2.23 (s, 3H; Ac), 2.14 (s, 6H; 2Ac), 2.08 (s, 9H; 3Ac), 2.06 (s, 3H; Ac), 2.04 (s, 3H; Ac), 1.97 (s, 3H; Ac), 1.96 (s, 3H; Ac), 1.95 (s, 3H; Ac), 1.49 (t, $J_{gem} = 11.2$ Hz, 1H; H^c-3a), 1.28 (d, $J = 6.8$ Hz, 3H; CH^f_3), 1.22 (s, 9H; *t*Bu); ^{13}C NMR ($CDCl_3$, 100.6 MHz): $\delta = 176.50$ (C=O), 176.00 (C=O), 175.50 (C=O), 172.85 (C=O), 172.18 (C=O), 172.07 (C=O), 171.97 (C=O), 171.78 (C=O), 171.67 (C=O), 135.88, 124.72, 102.82, 100.98, 100.60, 100.37, 99.97, 98.18, 75.94, 75.43, 75.37, 74.12, 73.67, 73.03, 72.83, 72.20, 71.87, 71.60, 71.52, 71.21, 70.94, 70.66, 70.05, 69.91, 69.13, 69.00, 68.73, 68.47, 68.22, 67.84, 66.53, 63.60, 62.22, 61.81, 57.83, 57.22, 55.60, 53.78, 50.53, 39.75, 27.74 (3CH_3), 23.21 (Ac), 21.90 (Ac), 21.87 (Ac), 21.37 (Ac), 21.08 (Ac), 20.96 (Ac), 20.91 (Ac), 20.89 (Ac), 20.80 (Ac), 20.68 (Ac), 16.88 (CH_3); elemental analysis calcd (%) for $C_{72}H_{71}O_{19}NS$: C 67.17, H 5.64, N 1.09, S 2.49; found C 67.09, H 5.81, N 1.00, S 2.49.

Deprotected hexasaccharide 3: *Procedure A*: LiI (500 mg, 3.25 mmol) was added to a solution of compound **36** (201 mg, 84 μmol) in dry pyridine (5 mL). The mixture was stirred at 120 to 125°C for 8–10 h under N_2 atmosphere. The dark yellow solution was evaporated to dryness, co-evaporated with toluene to a corresponding carboxylic acid as a dark yellow amorphous solid which was directly used for the next reaction. A solution of the above in methanol (15 mL) was added NH_2-NH_2·H_2O (3 mL) solution for 4 h at 80 to 85°C. The mixture was concentrated under reduced pressure co-evaporated with toluene then acetylated with acetic anhydride/pyridine 1:1 in the presence of catalytic amount of DMAP at room temperature for overnight. The acetylated mixture was concentrated under reduced pressure to a crude product which was passed through a short column of silica gel eluted with dichloromethane/methanol 10:1 to give a bright yellow film. To a solution of this bright yellow film in methanol/water (2 mL, 1:1), was added 1M sodium methoxide (100 μL) and the mixture was stirred at room temperature for 48 h. The reaction mixture was then concentrated under reduced pressure to a crude mixture which was applied to a short column of silica gel eluted with nC_3H_7OH/HOAc/H_2O 6:1:1 to give a white solid. A mixture of this white solid, methanol/acetic acid (10 mL, 1:1) and 10% Pd/C (300 mg) was stirred at room temperature overnight under hydrogen atmosphere. The solids were filtered off and the filtrate concentrated to a crude product which was passed through a short column of silica gel and eluted with nC_3H_7OH/HOAc/H_2O 1:1:1 to give a pure compound **3** (15 mg, 35.0%) as an amorphous solid.

Procedure B: Compound **36a** (50 mg) was acetylated with acetic anhydride/pyridine 1:1 (10 mL) at room temperature overnight. The reaction mixture was evaporated under reduced pressure to a crude mixture which was passed through a short column of silica gel eluted with dichloromethane/methanol 10:1 to give an amorphous solid. LiI (100 mg, 0.75 mmol) was added to a solution of the above in dry pyridine (3 mL). The mixture was refluxed at 120 to 125°C for 6–8 h under N_2 atmosphere. The dark yellow solution was evaporated to dryness, co-evaporated with toluene (3 × 10 mL) and concentrated to the corresponding carboxylic acid as a dark yellow amorphous solid directly used for the next reaction. A solution of the free acid in methanol (7.5 mL), was treated with NH_2-NH_2·H_2O (2.5 mL) solution for 4 h at 80 to 85°C. The mixture was concentrated under reduced pressure, co-evaporated with toluene (3 × 10 mL) and acetylated with acetic anhydride/pyridine in the presence of a catalytic amount of DMAP at rt overnight. The mixture was concentrated and passed through a short column of silica gel eluted with dichloromethane/methanol 10:1 to give a bright yellow film. To a solution of this bright yellow film in methanol/water (1.5 mL, 1:1), was added a catalytic amount of 1M sodium methoxide (50 μL) and the solution was stirred at room temperature for 48 h. It was then concentrated under reduced pressure to give a product, which was applied to a short column of silica gel and eluted with nC_3H_7OH/HOAc/H_2O 1:1:1 to give a pure compound **3** (10 mg) in total 39% yield. $R_f = 0.26$ (nC_3H_7OH/HOAc/H_2O 1:1:1); ^{1}H NMR (D_2O, 600 MHz): $\delta = 5.12$ (d, $J_{1,2} = 3.6$ Hz, 1H; H^f-1), 4.83 (dd, 1H; H^f-5), 4.78 (d, $J_{1,2} = 3.0$ Hz, 1H; H^a-1), 4.60 (d, $J_{1,2} = 8.4$ Hz, 1H; H^d-1), 4.56 (d, $J_{1,2} = 7.8$ Hz, 1H; H^b-1), 4.54 (d, $J_{1,2} = 7.8$ Hz, 1H; H^e-1), 4.39 (s, 3H; H^d-6b, H^d-6a, H^b-4), 4.32–4.30 (m, 1H; H^a-2), 4.21 (s, 3H; H^d-6b, H^d-6a, H^a-4), 4.10-4.00 (m, 5H; H^a-5, H^e-3, H^a-6b, H^d-4, H^a-3), 3.95–3.56 (m, 25H; H^d-2, H^f-3, H^d-3, H^a-6a, H^c-6, H^f-4, H^c-5, H^c-4, H^f-2, H^b-3), 3.57–3.48 (m, 2H; H^b-2, H^e-2), 3.37 (s, 3H; OCH_3), 2.77 (dd, $J = 3.0$, 11.4 Hz, 1H; H^c-3e), 2.04 (s, 3H; Ac), 2.02 (s, 3H; Ac), 2.01 (s, 3H; Ac), 1.81 (t, $J_{gem} = 12.0$ Hz, 1H; H^c-3a), 1.20 (d, $J = 7.6$ Hz, 3H; CH^f_3); ^{13}C NMR (D_2O, 100.6 MHz): $\delta = 177.32$ (C=O), 173.14 (C=O), 173.05 (C=O), 172.95 (C=O), 103.41, 100.58, 100.47, 98.75, 97.54, 97.08, 76.26, 74.60, 73.91, 73.79, 73.68, 72.25, 71.95, 71.78, 71.42, 70.90, 70.75, 69.98, 69.68, 68.26, 68.19, 68.04, 67.78, 67.40, 67.69, 67.05, 66.74, 66.37, 65.66, 64.96, 62.26, 60.39, 59.89, 54.62, 53.91, 50.66, 47.50, 38.69 (CH_2), 21.23 (Ac), 21.05 (Ac), 21.00 (Ac), 14.17 (CH_3[f]); FABMS (*m*/*z*) (positive ion mode) calcd for $C_{46}H_{76}O_{36}N_3SNa_2$: 1324.5; found 1324.9 $[M + Na]^+$.

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[1] J.-M. Lo-Guidice, J.-M. Wieruszeski, J. Lemoine, A. Verbert, P. Roussel, G. Lamblin, *J. Biol. Chem.* **1994**, *269*, 18794.

[2] a) C. Capon, C. L. Laboisse, J. M. Wieruszeski, J. J. Maoret, C. Augeron, B. Fournet, *J. Biol. Chem.* **1992**, *267*, 19248; b) C. Capon,

  0947-6539/00/0618-3450 $ 17.50+.50/0

J.- M. Wieruszeski, J. Lemoine, J. C. Byrd, H. Leffler, Y. S. Kim, *J. Biol. Chem.* **1997**, *272*, 31957.
[3] S. Hemmerich, H. Leffler, S. D. Rosen, *J. Biol. Chem.* **1995**, *270*, 12035.
[4] a) K. C. Nicolaou, N. J. Bockovich, D. R. Carcanague, *J. Am. Chem. Soc.* **1993**, *115*, 8385; b) S. Tsuboi, Y. Isogai, N. Hada, J. K. King, O. Hindsgaul, M. Fukuda, *J. Biol. Chem.* **1996**, *271*, 27213; c) E. V. Chandrasekaran, R. K. Jain, K. L. Matta, *J. Biol. Chem* **1992**, *267*, 23806.
[5] a) Y. Ding, J. Labbe, O. Kanie, O. Hindsgaul, *Bioorg. Med. Chem.* **1996**, *4*, 683; b) Z. G. Wang, O. Hindsgaul, *Adv. Exp. Med. Biol.* **1998**, *435*, 219.
[6] a) R. K. Jain, R. Vig, R. D. Locke, A. Mohammad, K. L. Matta, *Chem. Commun.* **1996**, 65; b) R. K. Jain, R. Vig, R. Rampal, E. V. Chandrasekaran, K. L. Matta, *J. Am. Chem. Soc.* **1994**, *116*, 12123; c) A. Marra, P. Sinaÿ, *Carbohydr. Res.* **1990**, *195*, 303; d) A. Hasegawa, H. Ohki, T. Nagahama, H. Ishida, M. Kiso, *Carbohydr. Res.* **1991**, *212*, 277; e) W. Birberg, H. Loon, *Tetrahedron Lett.* **1991**, *32*, 7453; f) H. Kondo, Y. Ichikawa, C.-H. Wong, *J. Am. Chem. Soc.* **1992**, *114*, 8748; g) M. Wilstermann, L. O. Konow, U. Nilsson, A. K. Ray, G. Magnusson, *J. Am. Chem. Soc.* **1995**, *117*, 4742.
[7] a) A. K. Sarkar, K. S. Rostand, R. K. Jain, K. L. Matta, J. D. Esko, *J. Biol. Chem.* **1997**, *272*, 25608; b) M. J. Gaunt, J. Yu, J. B. Spencer, *J. Org. Chem.* **1998**, 63, 4172.
[8] S. David, S. Hanessian, *Tetrahedron*, **1985**, *41*, 643.
[9] R. K. Jain, B.-G. Huang, E. V. Chandrasekaran, K. L. Matta, *Chem. Commun.* **1997**, 23.
[10] T. Mukaiyama, Y. Murai, S. Shaoda, *Chem. Lett.* **1981**, 431.
[11] The detailed X-ray analysis of compound **10** and compound **21** will be published elsewhere.
[12] H. Lönn, *Carbohydr. Res.* **1985**, *139*, 105.
[13] S. Sato, M. Mori, Y. Ito, T. Ogawa, *Carbohydr. Res.* **1986**, *197*, C6.
[14] H. Lönn, *Carbohydr. Res.* **1985**, *139*, 135.
[15] R. K. Jain, C. F. Piskorz, E. V. Chandrasekaran, K. L. Matta, *Carbohydr. Res.* **1995**, *271*, 247.
[16] a) A. Kameyama, H. Ishida, M. Kiso, A. Hasegawa, *Carbohydr. Res.* **1991**, *209*, C1; b) K. C. Nicolaou, C. W. Hummel, Y. Iwabuchi, *J. Am. Chem. Soc.* **1992**, *114*, 3126; c) S. J. Danishefsky, J. Gervay, J. M. Peterson, K. McDonald, K. Koseki, T. Oriyama, D. A. Griffith, *J. Am. Chem. Soc.* **1992**, *114*, 8329; d) S. J. Danishefsky, K. Koseki, D. A. Griffith, J. Gervay, J. M. Peterson, F. E. McDonald, T. Oriyawa, *J. Am. Chem. Soc.* **1992**, *114*, 8331; e) A. Hasegawa, K. Fushimi, H. Ishida, M. Kiso, *J. Carbohydr.Chem.* **1993**, *12*, 1203; f) S. J. Danishefsky, J. Gervay, J. M. Peterson, F. E. McDonald, K. Koseki, D. A. Griffith, T. Oriyama, S. P. Marsdem, *J. Am. Chem. Soc.* **1995**, *117*, 1940; g) G. Kretzschmar, W. Stahl, *Tetrahedron* **1998**, *54*, 6341; h) H. Kondo, Y. Ichijawa, C.-H. Wang, *J. Am. Chem. Soc.* **1992**, *114*, 8748; i) S. DeFrees, F. Gaeta, Y.-C. Lin, Y. Ishikawa, C.-H. Wong, *J. Am. Chem. Soc.* **1993**, *115*, 7549; j) R. L. Halcomb, H. Huang, C.-H. Wong, *J. Am. Chem. Soc.* **1994**, *116*, 11315; k) J. S. Debenham, R. Madson, C. Roberts, B. Fraser-Reid, *J. Am. Chem. Soc.* **1995**, *117*, 3302; l) O. Seitz, C.-H. Wong, *J. Am. Chem. Soc.* **1997**, *119*, 8766; m) O. Blixt, T. Norberg, *J. Org. Chem.* **1998**, *63*, 2705; n) V. Ellervik, G. Magnussion, *J. Org. Chem.* **1998**, *63*, 9314.
[17] a) B. Liebe, H. Kunz, *Angew. Chem.* **1997**, *109*, 629; *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 618; b) J. M. Lassaletta, R. R. Schmidt, *Tetrahedron Lett.* **1995**, *36*, 4209; c) C. Unverzagt, *Tetrahedron Lett.* **1997**, *38*, 5627; d) V. Martichonok, G. M. Whitesides, *J. Am. Chem. Soc.* **1996**, *118*, 8187; e) Y. Ito, T. Ogawa, *Tetrahedron* **1990**, *46*, 89; f) K. C. Nicolaou, C. W. Hummel, W. J. Bockovich, C.-H. Wong, *J. Chem. Soc. Chem. Commun.* **1991**, 870; g) T. J. Martin, R. R. Schmidt, *Tetrahedron Lett.* **1992**, *33*, 6123; h) T. J. Martin, B. Brescello, A. Toepfer, R. R. Schmidt, *Glycoconjugate J.* **1993**, *10*, 16; i) M. Wilstermann, G. Magnusson, *J. Org. Chem.* **1995**, *62*, 7961; j) S. Fujita, M. Numata, M. Sugimoto, K. Tomita, T. Ogawa, *Carbohydr. Res.* **1994**, *263*, 181; k) J. C. Castro-Palomino, Y. E. Tsvetkov, R. R. Schmidt, *J. Am. Chem. Soc.* **1998**, *120*, 5434; l) N. Hossain, G. Magnusson, *Tetrahedron Lett.* **1999**, *40*, 2217; m) C. Gege, J. Vogel, G. Bendas, U. Rothe, R. R. Schmidt, *Chem. Eur. J.* **2000**, *6*, 111.
[18] a) S. Fujita, M. Numata, M. Sugimoto, K. Tomita, T. Ogawa, *Carbohydr. Res.* **1994**, *263*, 181; b) A. Demchenko, G.-J. Boons, *Tetrahedron Lett.* **1998**, *39*, 3065; c) A. Demchenko, G.-J. Boons, *Chem. Eur. J.* **1999**, *5*, 1278.
[19] K. Scheffler, B. Ernst, A. Katopodis, J. L. Magnani, W.-T. Wang, R. Weisemann, T. Peters, *Angew. Chem.* **1995**, *107*, 2034; *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 1841.
[20] a) K. Okamoto, T. Kondo, T. Goto, *Bull. Chem. Soc. Jpn.* **1987**, *60*, 637; b) H. Hori, T. Nakajima, Y. Nishida, H. Ohrui, *Tetrahedron Lett.* **1988**, *29*, 6317; c) S. Prytulla, J. Lauterwein, M. Klessinger, J. Thiem, *Carbohydr. Res.* **1991**, *215*, 345; d) T. Ercegovic, G. Magnusson, *J. Chem. Soc. Chem. Commun.* **1994**, 831; e) S. J. Danishefsky, J. Gervay, J. M. Peterson, F. E. McDonald, K. Koseki, D. A. Griffith, T. Oriyama, S. P. Marsden, *J. Am. Chem. Soc.* **1995**, *117*, 1940; f) T. Ehara, A. Kameyama, Y. Yamada, H. Ishida, M. Kiso, A. Hasegawa, *Carbohydr. Res.* **1996**, *281*, 237.
[21] a) Y. Oikawa, T. Yoshioka, O. Yinemitsu, *Tetrahedron Lett.* **1982**, *23*, 885.
[22] PMB group is an acidic labile group, for example, PMB ether could be cleaved by a) AgOTf, H. Huang, C.-H. Wong, *J. Org. Chem.* **1995**, *60*, 3100; b) hot acetic acid, K. J. Hodgetts, T. W. Wallace, *Synth. Commun.* **1994**, *24*, 1151; c) 0.5% TFA in dichloromethane, A. Bouzide, G. Sauve, *Tetrahedron Lett.* **1999**, *40*, 2883; d) R. K. Jain, K. L. Matta, *Tetrahedron Lett.* **1992**, *31*, 4325, however, the NAP group is stable in these conditions.
[23] a) H. Kunz, C. Unverzagt, *Angew. Chem.* **1988**, *99*, 1763; *Angew. Chem. Int. Ed. Engl.* **1988**, *27*, 1697; b) A. Dan, M. Lergenmüller, M. Amano, Y. Nakahara, T. Ogawa, Y. Ito, *Chem. Eur. J.* **1998**, *4*, 2182; c) A. Cappa, E. Marcantoni, E. Torregiani, G. Bartoli, M. C. Bellucci, M. Bosco, L. Sambri, *J. Org. Chem.* **1999**, *64*, 5696.
[24] C. Abeygunawardana, C. A. Bush, J. O. Cisar, *Biochemistry* **1991**, *30*, 8568.
[25] S. J. Angyal, V. A. Pickles, *J. Chem. Soc. Perkin Trans. 1* **1988**, 2383.

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