Chemoenzymatic Synthesis of Sialyl Oligosaccharides with Sialidases Employing Transglycosylation Methodology

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A series of sialyloligosaccharides was synthesized using the transglycolytic activity of the sialidases from *Vibrio cholerae, Clostridium perfringens, Salmonella typhimurium,* and Newcastle disease virus. According to their hydrolytic activities the sialidases from *V. cholerae* and *C. perfringens* catalyze preferentially the formation of sialyl α(2−6)-linkages whereas the sialidases from *S. typhimurium* and Newcastle disease virus show a distinct preference for α(2−3) directed sialylations. Using combined chemical and enzymatic methodologies structures such as T-(Thomsen−Friedenreich) antigen [β-D-Gal-(1−3)-α-D-GalNAc-OThr], Tn-(Thomsen nouveau) antigen (α-D-GalNAc-OThr) and β-D-Gal-(1−4)-α-D-2-deoxy-Gal-OMe were sialylated in α(2−3)- and α(2−6)-positions regioselectively or in high regioisomeric excess and purified by simple isolation procedures. Depending on the enzyme source and acceptor structure yields for transsialylation varied between 10 and 30%.

Introduction

Neuraminic acid is a major constituent in a variety of glycoconjugates such as glycoproteins, gangliosides and oligosaccharides occurring in higher animals, viruses, bacteria, protozoa, and pathogenic fungi.[1,2] The growing awareness of the crucial role sialylation plays in the regulation of cellular and molecular recognition in biological systems[3] has led to an increased interest in this area.

Variations in glycosylation patterns of cell surface glycoconjugates have been associated with cell differentiation and cell adhesion in the inflammatory processes,[4] malignant cell transformations,[5] and pathogenesis of various diseases[6] and are attractive targets for diagnosis and treatment. Most frequently *N*-acetylneuraminic acid can be found attached α(2−3) or α(2−6) to galactose, *N*-acetylgalactosamine and *N*-acetylglucosamine and α-(2−8) to another *N*-acetylneuraminic acid unit in terminal position.

To establish structure−reactivity relationships, procedures have to be developed that allow the stereoselective synthesis of sialylated oligosaccharides in sufficient quantities since isolation from natural sources has proved to be less effective and excludes access to nonnatural derivatives.

Despite considerable recent progress[7] classical chemical methods lack experimental simplicity and stereospecificity. Chemoenzymatic approaches employing sialyltransferases[8−10] and sialidases[11−13] as synthetic tools have offered an alternative access to the family of sialylated glycostructures with excellent stereospecificity and varying regioselectivity depending on the type and source of enzyme.

Pronounced acceptor specificity and the requirement for relatively complex donor substrates restrict the use of sialyltransferases for preparative-scale synthesis of a wide array of natural and nonnatural sialyl oligosaccharides.

The concept of using the reversible nature of glycosidases for the synthesis of various oligosaccharides has reached the status of a facile glycosylation procedure[14,15] and found extended use as a simple, cheap, and efficient method for cases in which the use of the respective transferase is limited due to one of the reasons mentioned above.

Especially the kinetically controlled transglycosylation approach has been shown to produce, by rational choice of enzyme source, acceptor structure, reaction conditions, high regioselectivity, and yields of up to 60%.[16] This is

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(1) Varki, A. *Glycobiology* **1993**, *3*, 97−130.
(2) Corfield, A. P.; Schauer, R. In *Sialic Acids-Chemistry, Metabolism and Function*; Schauer, R., Ed.; Springer: Wien, 1982; pp 5−50.
(3) Schauer, R.; Vliegenthart, J. F. G. In *Glycoproteins II*; Montreuil, J., Vliegenthart, J. F. G., Schachter, H., Eds.; Elsevier Science B. V.: Oxford, 1997; pp 243−402.
(4) Lasky, L. A. *Annu. Rev. Biochem.* **1995**, *64*, 113−139.
(5) Hakomori, S. *Curr. Opin. Immunol.* **1991**, *3*, 646−653.
(6) Rogers, G. N.; Herrier; G.; Paulson, J. C. *J. Biol. Chem.* **1986**, *261*, 5947−5951. Higa, H. H.; Rogers, G. N. Paulson, J. C. *Virology* **1985**, *144*, 279−282. Tavakkol, A.; Burness, A. T. H. *Biochemistry* **1990**, *29*, 10684−10690.
(7) Ito, Y.; Gaudino, J. J.; Paulson, J. C. *Pure Appl. Chem.* **1993**, *65*, 753−762. De Ninno, M. P. *Synthesis* **1991**, 583−593. Okamoto, K.; Goto, T. *Tetrahedron* **1990**, *46*, 5835−5857. Martichonok, V.; Whitesides, G. M. *J. Am. Chem. Soc.* **1996**, *118*, 8187−8191.
(8) Thiem, J.; Treder, W. *Angew. Chem., Intl. Ed. Engl.* **1986**, *25*, 1096−1097. Kren, V.; Thiem, J. *Angew. Chem., Intl. Ed. Engl.* **1995**, *34*, 893−895.
(9) Ichikawa, Y.; Shen, G. J. Wong, C. H. *J. Am. Chem. Soc.* **1991**, *113*, 4698−4700. Ichikawa, Y.; Liu, J. L. L.; Shen, G. J. Wong, C. H. *J. Am. Chem. Soc.* **1991**, *113*, 6300−6302.
(10) Lubineau, A.; Basset-Carpentier, K.; Augé, C. *Carbohydr. Res.* **1997**, *300*, 161−167.
(11) Thiem, J.; Sauerbrei, B. *Angew. Chem., Intl. Ed. Engl.* **1991**, *30*, 1503−1505.
(12) Ajisaka, K.; Fujimoto, H.; Isomura, M. *Carbohydr. Res.* **1994**, *259*, 103−115.
(13) Makimura, Y.; Ishida, H.; Kondo, A.; Hasegawa, A.; Kiso, M. *J. Carbohydr. Chem.* **1998**, *17*, 975−979.
(14) Kren, V.; Thiem, J. *Chem. Soc. Rev.* **1997**, *26*, 463−473.
(15) Murata, T.; Usui, T. *Biosci. Biotech. Biochem.* **1997**, *61*, 1059−1066.

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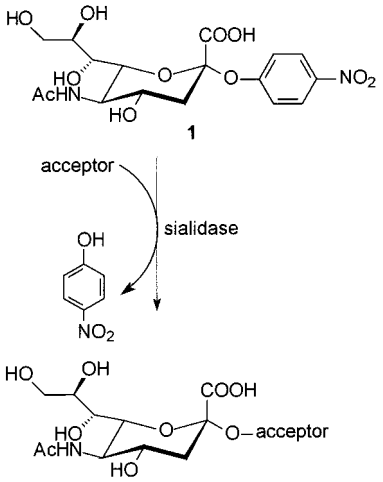
Scheme 1. Sialidase-Catalyzed Transsialylation



favorite competition to classical chemical procedures for which not only the glycosylation step itself but also the often numerous protection and deprotection steps have to be taken into account.

So far, the most successful transglycosylation procedures involved use of β-galactosidases from various sources due to their excellent synthetic potential considering yield, availability and regioselectivity. On a more limited scale, sialidases have also shown their applicability as useful tools for stereoselective sialylation.[11−13]

In contrast to the more demanding transferases that require expensive nucleotide sugars glycosidases need less complicated donors. In the case of sialidases—as for most glycosidases—the *p*-nitrophenyl glycoside is the donor of choice despite some solubility problems and feedback inhibition of the enzyme by liberated *p*-nitrophenol (*p*-NP-OH).

The major objective of this work was to demonstrate the feasibility of the transsialylation approach for the synthesis of various sialyl oligosaccharides and to develop a versatile toolbox for regioselective sialylation procedures that allow syntheses of a wide variety of natural and nonnatural sialyl oligosaccharides (Scheme 1).

By selecting glycosidases with different regioselectivities such as *Vibrio cholerae*, *Clostridium perfringens*, *Salmonella typhimurium*, and Newcastle disease virus sialidase synthetic access toward the two major naturally occurring glycosylation patterns, $\alpha(2-3)$- and $\alpha(2-6)$-sialylation, could be achieved.

Results and Discussion

Eight different acceptors were tested as potential acceptors for enzymatic transsialylation including epitopes of the T-(Thomsen−Friedenreich) antigen (**4**) and the Tn-(Thomsen-nouveau) antigen (**2**), which have received increasing attention since they were identified as tumor-associated antigens.[17] Furthermore, Tn and sialyl-Tn epitopes were discovered on the envelope glycoprotein gp120 of the HIV-virus.[18] In this work, the naturally occurring $\alpha(2-3)$-linked T- and Tn-epitopes were synthesized as well as the nonnatural $\alpha(2-6)$-linked epitopes.

Disaccharides **4**, **8**, and **12** were synthesized in a transglycosylation approach using β-galactosidases from different origins (Schemes 2−4).

Compounds **4** and **8** were prepared from **2** and **7**, respectively, by regioselective $\beta(1-3)$-transgalactosylation with β-galactosidase from bovine testes (Schemes 2 and 3), which yielded **4**[19] and **8** regioselectively in 21% and 24% yield, respectively. Disaccharide **12** was obtained as the only regioisomer from **11** in 36% yield catalyzed by β-galactosidase from *Bacillus circulans* (Scheme 4).

As has been shown in earlier work[11,12] the sialidases from *V. cholerae* and *C. perfringens* show a distinct preference for the formation of $\alpha(2-6)$ directed sialylations. At relatively low temperatures around 20−25 °C the transglycolytic activity is ambiguous.[20] Here the *V. cholerae* and *C. perfringens* sialidases display a preference for $\alpha(2-6)$- against $\alpha(2-3)$-sialylation of about 3:1 to 5:1 for most acceptor structures. At higher temperatures kinetic resolution is shifted in favor of the $\alpha(2-6)$ linkage although often at the cost of overall yield. At 37 °C only traces of $\alpha(2-3)$ linked products can be detected by HPLC.

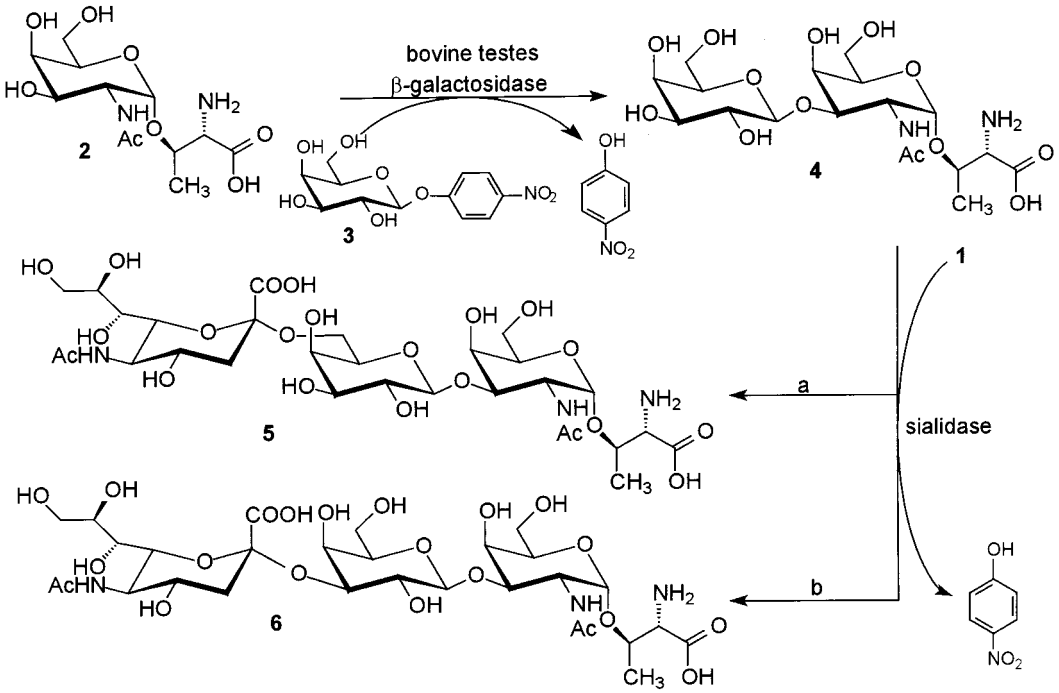
Newcastle disease virus sialidase is known for its specificity for the $\alpha(2-3)$ linkage and has been used by Ajisaka et al. for regioselective $\alpha(2-3)$-transsialylation.[12] *S. typhimurium* sialidase is the first known bacterial sialidase with a distinct regioselectivity for $\alpha(2-3)$ linkages.[21] The synthetic potential of this sialidase for $\alpha(2-3)$ sialyl structures has already been indicated.[13]

For all four sialidases two factors seemed crucial for the success of the transglycosylation reaction and pronounced regioselectivity: temperature and incubation time. When optimizing the incubation conditions and taking earlier results into account it showed that low temperatures promoted adjustment of the regioselectivities according to the hydrolytic abilities of the enzymes. This holds especially true for the sialidases from *V. cholerae* and *C. perfringens*. At lower temperatures, mixtures of $\alpha(2-3)$- and $\alpha(2-6)$-linked products were obtained whereas at higher temperatures the regioisomeric ratio is shifted in favor of the $\alpha(2-6)$ product if the reaction is carefully monitored and the incubation is interrupted at the peak of product formation. For the sialidase from Newcastle disease virus which has a significantly higher specificity for the $\alpha(2-3)$ regioisomer no such influences could be detected. The *S. typhimurium* sialidase displays a transglycolytic regioselectivity slightly more susceptible to temperature than Newcastle disease virus sialidase but not as pronounced as the *V. cholerae* and *C. perfringens* sialidases.

The course of the transsialylation reaction was routinely monitored by TLC and photometrically by detection of liberated *p*-NP-OH. To study kinetically induced regioisomeric shifts of transsialylation the reactions were monitored by HPLC. After 80% turnover of donor the incubation was stopped in order to minimize product hydrolysis.

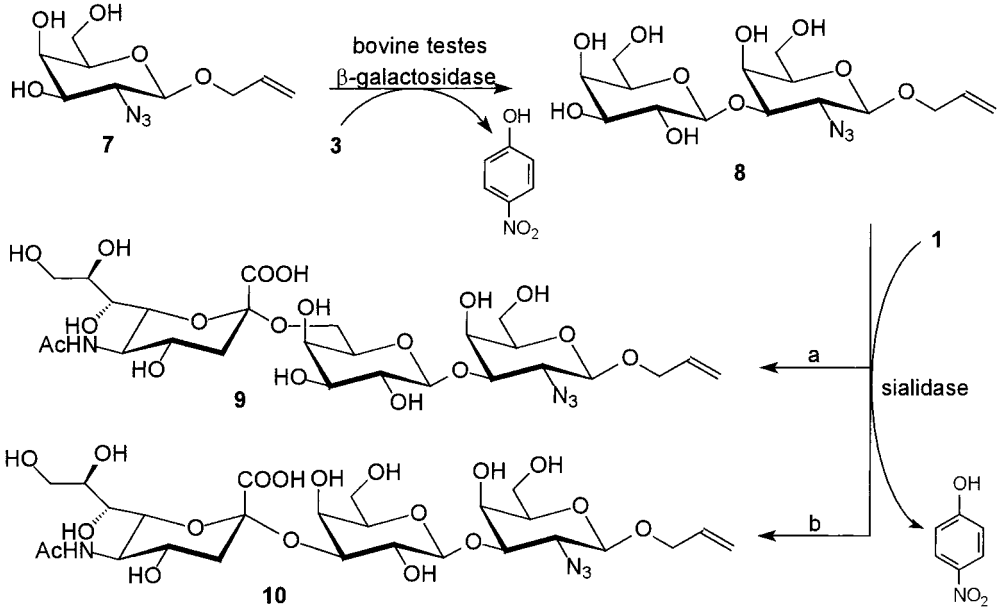
(16) Farkas, E.; Thiem, J. *Eur. J. Org. Chem.* **1999**, 3073−3077.
(17) Springer, G. F. *Science* **1984**, *224*, 1198−1206. Hakomori, S. *Adv. Cancer. Res.* **1989**, *52*, 257−331. Hull, S. R. Bright, A.; Carraway, K. L. Abe, M.; Hayes, D. F.; Kufe, D. W. *Cancer. Commun.* **1989**, *1*, 261−267. Hakomori, S. In *Glycoproteins and Disease*; Montreuil, J., Vliegenthart, J. F. G., Schachter, H., Eds.; Elsevier Science B. V.: Oxford, 1996; pp 243−275.
(18) Hansen, J.-E. S.; Clausen, H.; Hu, S. L.; Nielsen, J. O.; Olofsson, S. *Arch. Virol.* **1992**, *126*, 11−20.
(19) Gambert, U.; Thiem, J. *Carbohydr. Res.* **1997**, *299*, 85−89.
(20) Sauerbrei, B.; Schmidt, D.; Thiem, J. Unpublished results.
(21) Hoyer, L. L.; Roggentin, P.; Schauer, R.; Vimr, E. R. *J. Biochem. (Tokyo)* **1991**, *110*, 462−467.

Scheme 2. Sialidase-Catalyzed Synthesis of Sialyl T-antigen Isomers 5 and 6[a]



[a] Key: (a) *Vibrio cholerae* sialidase or *Clostridium perfringens* sialidase; (b) *Salmonella typhimurium* sialidase or Newcastle disease virus sialidase.

Scheme 3. Sialidase-Catalyzed Synthesis of Sialyl T-Antigen Precursors 9 and 10[a]



[a] Key: (a) *Vibrio cholerae* sialidase or *Clostridum perfringens* sialidase; (b) *Salmonella typhimurium* sialidase or Newcastle disease virus sialidase.

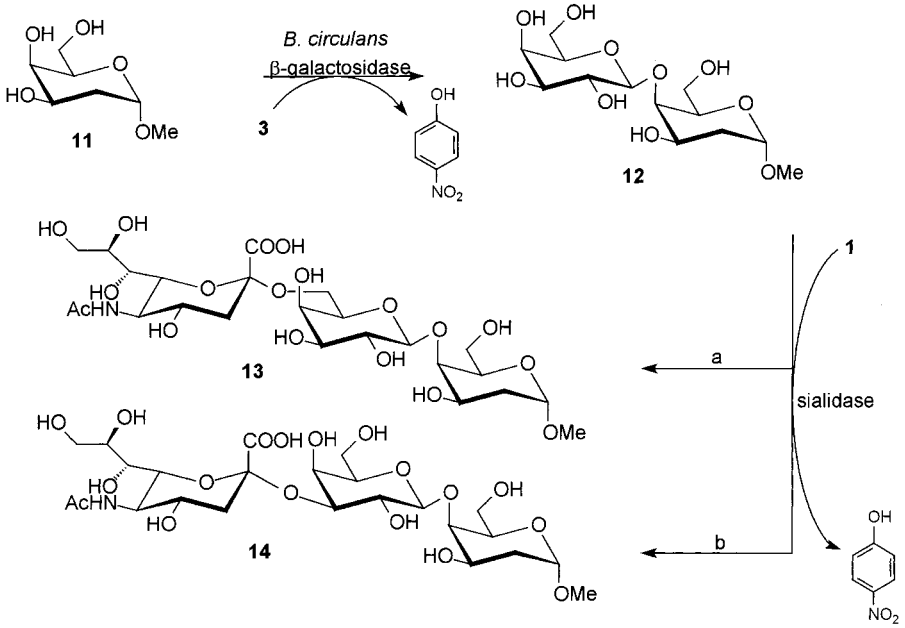
Structural information of the transsialylation products was preferentially obtained by ^{13}C NMR spectroscopy as the congested region between 3.5 and 4.5 ppm in the ^{1}H NMR spectrum did not allow complete unequivocal assignment. Significant downfield shifts of 6 to 10 ppm at the glycosidic linkage position of the acceptor molecule as well as minor shift changes in the linked donor molecule of 0.1−0.5 ppm were representative for the regio- and stereoselectivity of the glycosyl transfer. Regioisomeric transfer ratios can be determined by integration of the axial and equatorial H-3-protons of neuraminic acid applying the structural-reporter-group concept.[22] The shifts of the *N*-acetylneuraminic acid H-3-protons not only are located outside the bulk signal region but also contain information about the type and linkage of the glycosidically coupled saccharide.

Depending on the source of the sialidase and by optimized incubation conditions a fairly good regioselectivity could be achieved. The results are summarized in Table 1.

All *V. cholerae* and *C. perfringens* sialidase catalyzed transsialylations were performed at 30 °C. This temper-

(22) Vliegenthart, J. F. G.; Dorland, L.; von Halbeek, H. *Adv. Carbohydr. Chem. Biochem*. **1983**, *41*, 209−374.

Scheme 4. Sialidase-Catalyzed Synthesis of Sialylated Trisaccharides 13 and 14[a]



[a] Key: (a) *Vibrio cholerea* sialidase or *Clostridium perfringens* sialidase; (b) *Salmonella typhimurium* sialidase or Newcastle disease virus sialidase.

Table 1. Sialidase-Catalyzed Transsialylation: Yields and Regioisomeric Ratios

glycosyl acceptor	sialidase	regioisomeric ratios[a] α(2−6)	α(2−3)	main product	total yield (%)
2	VC[b]	>99	<1	26	16
	CP[c]	>99	<1	26	10
	ST[d]	5	95	27	15
	NDV[e]	<1	>99	27	10
4	VC[b]	>99	<1	5	12
	CP[c]	>99	<1	5	4
	ST[d]	7	93	6	11
	NDV[e]	<1	>99	6	10
8	VC[b]	>99	<1	9	12
	CP[c]	>99	<1	9	7
	ST[d]	12	88	10	11
	NDV[e]	<1	>99	10	11
11	VC[b]	>99	<1	22	26
	CP[c]	>99	<1	22	15
	ST[d]	8	92	23	22
	NDV[e]	<1	>99	23	14
12	VC[b]	90	10	13	15
	CP[c]	>99	<1	13	5
	ST[d]	14	86	14	10
	NDV[e]	<1	>99	14	8
15	VC[b]	>99	<1	18	18
	CP[c]	>99	<1	18	10
	ST[d]	5	95	19	16
	NDV[e]	<1	>99	19	17
16	VC[b]	>99	<1	20	16
	CP[c]	>99	<1	20	10
	ST[d]	<1	>99	21	15
	NDV[e]	<1	>99	21	18
17	VC[b]	>99	<1	24	21
	CP[c]	>99	<1	24	14
	ST[d]	<1	>99	25	20
	NDV[e]	<1	>99	25	14

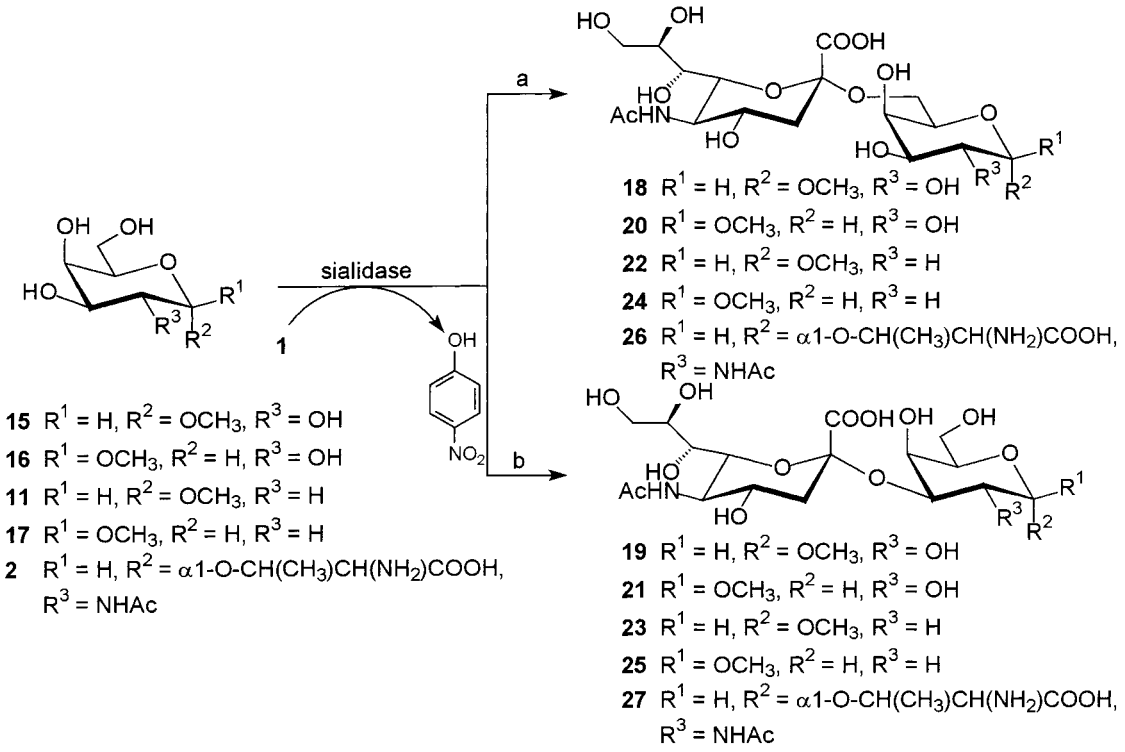
[a] Determined by HPLC.[29] [b] *Vibrio cholerae* sialidase. [c] *Clostridium perfringens* sialidase. [d] *Salmonella typhimurium* sialidase. [e] Newcastle disease virus sialidase.

ature represents the best compromise regarding yield and regioselectivity. When the course of the reaction reaches a maximum of α(2−6) product only traces of the α(2−3) products are detected. By prolonged reaction times these minor side products can in most cases be eliminated although at the cost of total yield. For *S. typhimurium* sialidase 30 °C proved to be the most suitable temperature whereas Newcastle disease virus sialidase tolerated an incubation temperature of 37 °C without detectable formation of undesired α(2−6) isomers. To retain enzyme stability bovine serum albumin (BSA) was added to the enzyme stock solution.

V. cholerae sialidase proved to be the most efficient glycosidase for the synthesis of sialyl α(2−6)-linkages. Except for disaccharide **12** all acceptor structures were regioselectively sialylated in α(2−6)-position in a yield of 12−26%. The sialylated derivatives of T-antigen (**4**) and T-antigen precursor **8** were synthesized in 12% (Schemes 2 and 3), trisaccharide **9** in 15% (Scheme 4). Slightly higher yields were obtained for transsialylation of the monosaccharide acceptors **2** (16%), **11** (26%), **15** (18%), **16** (16%), and **17** (21%) (Scheme 5). The formation of α(2−3)-linked regioisomers could be mostly avoided by variation of incubation time although sometimes at the cost of overall yield. But as chromatographic separation of the respective regioisomers posed considerable problems it was preferable to optimize the regioisomeric excess in favor of the overall yield. *C. perfringens* sialidase catalyzed exclusive sialylation at the C-6 position with all tested acceptors. Yields were in a range from 4 to 15% and, therefore, considerably lower than for *V. cholerae* sialidase. The trisaccharides **5**, **9**, and **13** were obtained in 4, 7, and 5%, respectively (Schemes 2−4). The monosaccharide acceptors **2**, **11**, and **15**−**17** showed, as with *V. cholerae* sialidase, slightly better transsialylation results (10−15%, Scheme 5). Furthermore, longer incubation times were necessary.

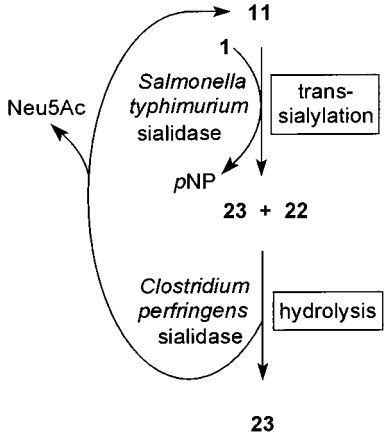
The predominant synthesis of α(2−3)-sialyloligosaccharides was achieved using commercially available *S. typhimurium* sialidase. Disaccharides **4**, **8**, and **12** could be sialylated in 10−11% (Schemes 2−4) whereas transsialylation of **2**, **15**, and **16** proceeded in 15−16% (Scheme 5). Even higher yields of 20 and 22%, respectively, were observed for sialylation of acceptors **11** and **17** (Scheme 5). All acceptors were sialylated at C-3 in high regioisomeric excess. Compounds **16** and **17** were sialylated regiospecifically in α(2−3)-position. To obtain pure α(2−

Scheme 5. Sialidase-Catalyzed Synthesis of Various Sialylated Disaccharides 18−27[a]



[a] Key: (a) *Vibrio cholerae* sialidase or *Clostridium perfirngens* sialidase; (b) *Salmonella typhimurium* sialidase or Newcastle disease virus sialidase.

Scheme 6. Sialidase-Catalyzed Regiospecific Hydrolisis of Isomeric Byproducts



3)-linked products an enzymatic postreaction was applied, an approach which was previously described by Hedbys et al. for *β*-galactosidase catalyzed transglycolytic synthesis of galactosylated oligosaccharides.[23] To obtain a regiospecifically pure product the undesired regioisomer in a product mixture was selectively hydrolyzed by incubation with a glycosidase with the respective regioselectivity (example shown in Scheme 6). In this case the reaction mixture was, after denaturation of *S. typhimurium* sialidase, diluted with further incubation buffer. Then, 10 mU of *C. perfringens* sialidase was added, and the mixture was incubated at 25 °C. Undesired α(2−6)-linked isomers were selectively hydrolyzed within 1 min. Competition experiments showed only minor loss of α(2−3)-linked product in the course of this treatment. But since kinetic resolution of the respective regioisomers is not very distinct extreme care had to be taken that the incubation was immediately stopped after hydrolysis of the undesired regioisomer.

Newcastle disease virus sialidase displayed a high regiospecificity for the synthesis of α(2−3)-linkages. All acceptors were selectively α(2−3) sialylated, although, compared with *S. typhimurium* sialidase, with slightly lower yields of 8−18%. The sialyl compounds **6**, **10**, **14**, and **27** could be synthesized in 8−11% (Schemes 2−5) whereas acceptors **11** and **15**−**17** were sialylated in 14−18% yield (Scheme 5).

A limiting factor to all transsialylations described is the necessity for using an excess of acceptor substrate of 5:1 to 7:1 to achieve optimum yields. In contrast to, e.g., *β*-galactosidases an increase in the amount of donor used in transglycosylation leads to a significant decrease in yields. Compound **24**, for instance, was synthesized using a donor/acceptor ratio of 1:1.5 in 13% yield, which corresponds to a transfer efficiency of only 62% compared to the optimized procedure. Therefore, every transsialylation needs to be adapted individually to the respective circumstances either in favor of the yield or taking a limited amount of acceptor into account. Unreacted acceptor, though, is usually recovered quantitatively in the course of the workup procedure.

Conclusion

In general, it could be observed that the regioselectivity of all sialidases was sufficiently high for transglycolytic syntheses of sialyloligosaccharides with a defined linkage position. Regioisomeric side products could be selectively hydrolyzed with a sialidase of the respective regioselectivity. A further general trend was the decrease of overall yield with increasing size of acceptors. Depending on the

(23) Hedbys, L.; Johansson, E.; Mosbach, K.; Larsson, P.-O. *Carbohydr. Res.* **1989**, *186*, 217−223.

enzyme source 10−50% higher yields were obtained with the relatively small monosaccharide acceptors than with the sterically more demanding disaccharides.

Comparing the two enzymatic approaches for sialylation—the use of sialyltransferases and sialidases—the transferases have the advantage of higher yields and regiospecific product formation but are limited by their acceptor specificity. As regards costs, simplicity and versatility of acceptors sialidases can offer a promising alternative for the synthesis of sialylated glycostructures. Therefore, the employed sialidases can be regarded as a useful toolbox for various sialylation problems.

Experimental Section

General Procedures. Methods. ^{1}H (400 and 500 MHz) and ^{13}C NMR (100.67 and 125.77 MHz) spectra were recorded at ambient temperature with samples in D_2O. As an internal standard for ^{13}C NMR spectra acetonitrile ($\delta_{CH3} = 1.47$) was used. Peak assignments were confirmed by DEPT and ^{1}H^{1}H-, ^{1}H^{13}C-COSY-, and TOCSY-experiments. Thin-layer chromatography was performed on precoated silica gel plates (60-F254, Merck). Products were visualized by heating to 100 °C after spraying with resorcinol/HCl[24] (sialyl oligosaccharides) or charring after spraying with 10% sulfuric acid in ethanol (neutral oligosaccharides).

Sialidase (E.C. 3.2.1.18) from *V. cholerae* was a kind gift from Chiron Behring GmbH, Germany. Sialidase (E.C. 3.2.1.18) from *C. perfringens* was a kind gift from Boehringer Mannheim GmbH, Germany. Sialidase (E.C. 3.2.1.18) from Newcastle disease virus was purchased from Oxford Glycosystems Ltd., U.K. Recombinant sialidase (E.C. 3.2.1.18) from *S. typhimurium* was purchased from New England Biolabs GmbH, Germany. β-Galactosidase (E.C. 3.2.1.23) from *B. circulans* was a kind gift from Daiwa Kasei Co., Ltd., Japan. Methyl α-and β-D-galactopyranoside and *p*-nitrophenyl β-D-galactopyranoside were purchased from Sigma, Germany.

2-*O*-(*p*-Nitrophenyl)-5-acetamido-3,5-dideoxy-D-glycero-α-D-*galacto*-nonulopyranusonic acid (*p*-NP-Neu5Ac, **1**)[25] and acceptors **2**,[19] **7**,[26] **11**,[27] and **16**[27] were prepared by established procedures.

General Transsialylation Procedure. Depending on the source of the sialidase, solutions of *p*-NP-Neu5Ac (**1**) and the respective acceptor were incubated in a ratio from 1:5 to 1:7 in degassed incubation buffer with the respective sialidase. Incubation time, buffer, and temperature depended on the source of sialidase (see Table 1 and specific procedures).

The course of the reaction was followed photometrically at 400 nm and by TLC (ethanol/1 M ammonium acetate (pH 7.4) 5:1). After reaction was complete, the incubation was interrupted by heating at 90 °C for 6 min. The mixture was acidified to pH 6.8 with Dowex 50WX8 H^+ cation exchanger and centrifuged. The supernatant was decanted, diluted with distilled water to a final volume of 5 mL, and applied to a column (1.1 × 19 cm) of Dowex 1 × 2 (phosphate form). Unreacted acceptor was washed off with distilled water (200 mL). Fractions of 5 mL were eluted using 5 mM sodium phosphate buffer (pH 6.8) and tested for sialic acid content with resorcinol spraying reagent.[25] Product fractions were pooled, lyophilized, dissolved in distilled water (2 mL), applied to a Biogel P2 column (16 × 900 mm), and eluted with distilled water. Unreacted acceptor was recovered quantitatively. Sialyloligosaccharide-containing fractions were pooled and lyophilized. NMR samples were repeatedly dissolved in D_2O and lyophilized before measurement.

Transsialylation Assay Using *C. perfringens* Sialidase. Solutions of 50 μmol of *p*-NP-Neu5Ac (**1**) and 350 μmol of acceptor (donor/acceptor ratio 1:7) in 250 μL of degassed incubation buffer (0.1 M NaOAc, 0.5 mM $CaCl_2$, pH 5.1) were incubated with 1 U of *C. perfringens* sialidase at 37 °C following the general transsialylation procedure.

Transsialylation Assay Using *V. cholerae* Sialidase. Solutions of 50 μmol of *p*-NP-Neu5Ac (**1**) and 350 μmol of acceptor (donor/acceptor ratio 1:7) in 250 μL of degassed incubation buffer (0.1 M NaOAc, 0.5 mM $CaCl_2$, pH 5.5) were incubated with 1 U of *V. cholerae* sialidase at 30 °C following the general transsialylation procedure.

Transsialylation Assay Using *S. typhimurium* Sialidase. Solutions of 50 μmol of *p*-NP-Neu5Ac (**1**) and 250 μmol of acceptor (donor/acceptor ratio 1:5) in 250 μL of degassed incubation buffer (0.1 M NaOAc, pH 5.1) were incubated with 1 U of *S. typhimurium* sialidase at 30 °C following the general transsialylation procedure.

Transsialylation Assay Using Newcastle Disease Virus Sialidase. Solutions of 50 μmol of *p*-NP-Neu5Ac (**1**) and 250 μmol of acceptor (donor/acceptor ratio 1:5) in 250 μL of degassed incubation buffer (0.1 M NaOAc, pH 5.5) are incubated with 1 U of Newcastle disease virus sialidase at 37 °C following the general transsialylation procedure.

Selective Sialidase-Catalyzed Hydrolysis of Regioisomeric Byproducts. After a completed transsialylation reaction, the incubation was interrupted by heating at 90 °C for 6 min. The mixture was then diluted with further incubation buffer to a volume of 600 μL. Then, 10 mU of *C. perfringens* sialidase (for hydrolysis of α(2−6)-linked byproducts) or Newcastle disease virus sialidase (for hydrolysis of α(2−3)-linked byproducts) was added, and the mixture was incubated at 25 °C for 1 min. To quench the reaction, 400 μL of ethanol was added, and the mixture was heated at 90 °C for 5 min (reaction vessel should be open). Further purification proceeded in analogy to the general procedure.

Methyl 2-Deoxy-4-*O*-(β-D-galactopyranosyl)-α-*lyxo*-hexopyranoside (12). Galactoside **11** (140 mg, 0.79 mmol, 1 equiv) and donor **3** (722 mg, 2.4 mmol, 3 equiv) were incubated in 3.8 mL of 50 mM sodium acetate buffer (pH 5.0) with 1 U of β-galactosidase from *B. circulans* at 25 °C. The reaction was stopped after 28 h by addition of 5 mL of MeOH and 5 min of heating at 90 °C. Free *p*-NP-OH was separated by extraction with ethyl acetate. The mixture was lyophilized, diluted with distilled water to a final volume of 2 mL, applied to a Biogel P2 column (16 × 900 mm), and eluted with water. Product-containing fractions were pooled and lyophilized to a white foam (96 mg, 36% yield): mp 164−165 °C dec; $[\alpha]^{20}_D$ + 27° (*c* 0.6, H_2O); ^{1}H NMR (D_2O) δ 5.11 (dd, $J_{1,2eq}$ 0.9 Hz, $J_{1,2ax} = 3.6$ Hz, 1 H, *H*-1), 4.50 (d, $J_{1',2'} = 7.8$ Hz, 1 H, *H*-1′), 4.16 (ddd, $J_{3,4} = 2.8$ Hz, 1 H, *H*-3), 4.09 (bd, $J_{4,5} = 1.0$ Hz, 1 H, *H*-4), 3.88 (ddd, $J_{5,6a} = 6.4$ Hz, $J_{5,6b} = 6.6$ Hz, $J_{6a,6b} = 12.2$ Hz, 1 H, *H*-5), 3.81 (dd, $J_{3',4'} = 3.2$ Hz, 1 H, *H*-4′), 3.72 (m, 2 H, *H*-6a, *H*-6b), 3.67 (m, 2 H, *H*-6a′, *H*-6b′), 3.68 (m, 1 H, *H*-5′), 3.57 (dd, $J_{2',3'} = 10.0$ Hz, 1 H, *H*-3′), 3.54 (s, 3 H, OCH_3), 3.48 (dd, 1 H, *H*-2′), 2.00−1.96 (m, 2 H, *H*-2ax, *H*-2eq); ^{13}C NMR (D_2O) δ 104.32 (C-1′), 98.74 (C-1), 75.84 (C-4), 75.21 (C-5′), 73.23 (C-3′), 71.56 (C-2′), 71.21 (C-5), 68.94 (C-4′), 68.25 (C-3), 67.88 (C-6), 61.83 (C-6′), 54.79 (OCH_3), 31.48 (C-2). Anal. Calcd for $C_{13}H_{24}O_{10}$: C 45.88, H 7.11. Found: C 45.67, H 7.02.

Allyl 2-Azido-2-deoxy-3-*O*-(β-D-galactopyranosyl)-β-D-galactopyranoside (8). Compound **7** (170 mg, 0.84 mmol) and donor **3** (759 mg, 2.52 mmol, 3 equiv) were incubated in 3.5 mL of 50 mM McIlvaine buffer (pH 4.3) with 1.6 U of β-galactosidase from bovine testes at 37 °C. The reaction was stopped after 48 h by addition of 5 mL of MeOH and 5 min of heating at 90 °C. Free *p*-NP-OH was separated by extraction with ethyl acetate. The mixture was lyophilized, diluted with distilled water to a final volume of 2 mL, applied to a Biogel P2 column (16 × 900 mm), and eluted with water. Product-containing fractions were pooled and lyophilized to a white foam (74 mg, 24% yield): mp 171−172 °C; $[\alpha]^{25}_D$ −14° (*c* 0.8, H_2O) (lit.[27] mp 168−170 °C; $[\alpha]^{25}_D$ −19° (*c* 1.0, H_2O)). NMR spectra were identical to the ones published.[27] Anal. Calcd for $C_{18}H_{31}NO_{13}$: C 46.05, H 6.66, N 2.98. Found: C 46.16, H 6.48, N 3.03.

Methyl (5-Acetamido-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosylonic acid)-(2−6)-α-D-galactopyranoside (18). Compounds **1** and **15** were incubated for 20 (*V.*

(24) Schauer, R. *Methods Enzymol.* **1978**, *50*, 64−89.
(25) Rothermel, J.; Faillard, H. *Carbohydr. Res.* **1990**, *196*, 29−40.
(26) Sabesan, S.; Lemieux, R. U. *Can. J. Chem.* **1984**, *62*, 644−654.
(27) Sabesan, S.; Neira, S. *J. Org. Chem.* **1991**, *56*, 5468−5472.

cholerae sialidase) and 24 h (*C. perfringens* sialidase) following the respective transsialylation procedures to give 4.4 mg (18%, *V. cholerae* sialidase) and 2.4 mg (10%, *C. perfringens* sialidase) of compound **18**, respectively. Analytical data were identical to published results.[11]

Methyl (5-Acetamido-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosylonic acid)-(2−3)-α-D-galactopyranoside (19). Compounds **1** and **15** were incubated for 24 (*S. typhimurium* sialidase) and 20 h (Newcastle disease virus sialidase) following the respective transsialylation procedures to give 3.9 mg (16%, *S. typhimurium* sialidase) and 4.1 mg (17%, Newcastle disease virus sialidase) of compound **19**, respectively. Analytical data were identical to published results.[11]

Methyl (5-Acetamido-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosylonic acid)-(2−6)-β-D-galactopyranoside (20). Compounds **1** and **16** were incubated for 20 (*V. cholerae* sialidase) and 24 h (*C. perfringens* sialidase) following the respective transsialylation procedures to give 3.9 mg (16%, *V. cholerae* sialidase) and 2.4 mg (10%, *C. perfringens* sialidase) of compound **20**, respectively. Analytical data were identical to published results.[11]

Methyl (5-Acetamido-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosylonic acid)-(2−3)-β-D-galactopyranoside (21). Compounds **1** and **16** were incubated for 24 (*S. typhimurium* sialidase) and 20 h (Newcastle disease virus sialidase) following the respective transsialylation procedures to give 3.6 mg (15%, *S. typhimurium* sialidase) and 4.5 mg (18%, Newcastle disease virus sialidase) of compound **21**, respectively. Analytical data were identical to published results.[11]

Methyl (5-Acetamido-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosylonic acid)-(2−6)-2-deoxy-α-D-*lyxo*-hexopyranoside (22). Compounds **1** and **11** were incubated for 24 (*V. cholerae* sialidase) and 36 h (*C. perfringens* sialidase) following the respective transsialylation procedures to give 6.1 mg (26%, *V. cholerae* sialidase) and 3.5 mg (15%, *C. perfringens* sialidase) of compound **22**, respectively: $[\alpha]^{20}_D$ +124° (*c* 0.8, H_2O); ^{1}H NMR (D_2O) δ 5.08 (dd, $J_{1,2eq}$ = 0.8 Hz, $J_{1,2ax}$ = 3.6 Hz, 1 H, *H*-1), 4.09 (d, $J_{3,4}$ = 3.6 Hz, 1 H, *H*-3), 4.01−3.97 (m, 2 H, H-5, H-6a), 3.94 (bd, $J_{4,5}$ = 1.0 Hz, 1 H, *H*-4), 3.89−3.86 (m, 2 H, H-8′, H-9a′), 3.82 (dd, $J_{5',6'}$ = 10.2 Hz, 1 H, *H*-5′), 3.72 (dd, $J_{6',7'}$ = 1.6 Hz, 1 H, *H*-6′), 3.65 (dd, 1 H, *H*-9b′), 3.65−3.61 (m, 2 H, *H*-6b, *H*-4′), 3.57 (dd, 1 H, *H*-7′), 3.53 (s, 3 H, OCH_3), 2.71 (dd, $J_{3eq',4'}$ = 4.8 Hz, $J_{3eq',3ax'}$ = 12.3 Hz, 1 H, *H*-3eq′), 1.96−1.92 (m, $J_{2ax,3}$ = 12.4 Hz, $J_{2eq,3}$ = 5.8 Hz, $J_{2ax,2eq}$ = 13.0 Hz, 2 H, *H*-2ax, *H*-2eq) 1.74 (t, $J_{3ax',4'}$ = 12.0 Hz, 1 H, *H*-3ax′); ^{13}C NMR (D_2O) δ 175.81 (*C*OCH_3′), 174.29 (C-1′), 100.68 (C-2′), 98.76 (C-1), 73.44 (C-6′), 72.54 (C-8′), 71.28 (C-5), 70.45 (C-6), 69.23 (C-4′), 69.20 (C-7′), 67.99 (C-3), 65.11 (C-4), 63.51 (C-9′), 54.76 (OCH_3), 52.71 (C-5′), 40.91 (C-3′), 31.72 (C-2), 22.91 (CO*C*H_3′). Anal. Calcd for $C_{18}H_{31}NO_{13}$: C, 46.05; H, 6.66; N, 2.98. Found: C, 45.41; H, 6.89; N, 2.91.

Methyl (5-Acetamido-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosylonic acid)-(2−3)-2-deoxy-α-D-*lyxo*-hexopyranoside (23). Compounds **1** and **11** were incubated for 24 (*S. typhimurium* sialidase) and 16 h (Newcastle disease virus sialidase) following the respective transsialylation procedures to give 5.2 mg (22%, *S. typhimurium* sialidase) and 3.3 mg (14%, Newcastle disease virus sialidase) of compound **23**, respectively: $[\alpha]^{20}_D$ +108° (*c* 1, H_2O); ^{1}H NMR (D_2O) δ 5.13 (dd, $J_{1,2eq}$ = 0.8 Hz, $J_{1,2ax}$ = 3.6 Hz, 1 H, *H*-1), 4.13 (ddd, $J_{3,4}$ = 3.2 Hz, 1 H, *H*-3), 4.07 (d, 1 H, *H*-4), 3.88−3.85 (m, 3 H, *H*-5′, *H*-8′, *H*-9a′), 3.68 (dd, $J_{4',5'}$ = 9.8 Hz, 1 H, *H*-4′), 3.65 (dd, $J_{6',7'}$ = 1.8 Hz, *H*-6′), 3.64 (dd, *H*-9b′), 3.60 (dd, $J_{7',8'}$ = 9.2 Hz, *H*-7′), 3.70−3.67 (m, 2 H, *H*-6a, *H*-6b), 3.54 (s, 3 H, OCH_3), 2.76 (dd, $J_{3eq',4'}$ = 4.8 Hz, $J_{3eq',3ax'}$ = 12.4 Hz, 1 H, *H*-3eq′), 2.00−1.96 (m, $J_{2ax,3}$ = 12.2, $J_{2eq,3}$ = 5.6 Hz, $J_{2ax,2eq}$ = 13.0 Hz, 2 H, *H*-2ax, *H*-2eq), 1.79 (t, $J_{3ax',4'}$ = 11.8 Hz, 1 H, *H*-3ax′); ^{13}C NMR (D_2O) δ 175.83 (*C*OCH_3′), 174.71 (C-1′), 100.28 (C-2′), 98.77 (C-1), 74.68 (C-3), 73.58 (C-6′), 72.59 (C-8′), 71.12 (C-5), 69.21 (C-4′), 68.96 (C-7′), 67.81 (C-6), 65.16 (C-4), 63.41 (C-9′), 54.72 (OCH_3), 52.53 (C-5′), 40.48 (C-3′), 31.68 (C-2), 22.86 (CO*C*H_3′). Anal. Calcd for $C_{18}H_{31}NO_{13}$: C, 46.05; H, 6.66; N, 2.98. Found: C, 45.74; H, 6.83; N, 2.82.

Methyl (5-Acetamido-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosylonic acid)-(2−6)-2-deoxy-β-D-*lyxo*-hexopyranoside (24). Compounds **1** and **17** were incubated for 20 (*V. cholerae* sialidase) and 36 h (*C. perfringens* sialidase) following the respective transsialylation procedures to give 4.9 mg (21%, *V. cholerae* sialidase) and 3.4 mg (14%, *C. perfringens* sialidase) of compound **24**, respectively: $[\alpha]^{20}_D$ −28° (*c* 0.7, H_2O); ^{1}H NMR (D_2O) δ 4.54 (dd, $J_{1,2eq}$ = 2.4 Hz, $J_{1,2ax}$ = 9.8 Hz, 1 H, *H*-1), 4.01 (dd, $J_{6a,6b}$ = 11.8 Hz, 1 H, *H*-6a), 3.95−3.86 (m, 4 H, *H*-8′, *H*-9a′, *H*-5′, *H*-4), 3.84 (ddd, $J_{3,4}$ = 3.2 Hz, 1 H, *H*-3), 3.76 (ddd, $J_{5,6a}$ = 8.0 Hz, $J_{5,6b}$ = 4.8 Hz, *H*-5), 3.70 (dd, $J_{6',7'}$ = 1.7 Hz, 1 H, *H*-6′), 3.64 (dd, 1 H, *H*-9b′), 3.61 (dd, $J_{4',5'}$ = 9.2 Hz, 1 H, *H*-4′), 3.56 (dd, $J_{7',8'}$ = 8.6 Hz, 1 H, *H*-7′), 3.54 (s, 3 H, OCH_3), 3.52 (dd, 1 H, *H*-6b), 2.72 (dd, $J_{3eq',4'}$ = 4.6 Hz, $J_{3ax',3eq'}$ = 12.4 Hz, 1 H, *H*-3eq′), 2.04 (ddd, $J_{2eq,3}$ = 5.2 Hz, $J_{2eq,2ax}$ = 12.0 Hz, 1 H, *H*-2eq), 1.74 (t, $J_{3ax',4'}$ = 12.2 Hz, 1 H, *H*-3ax′), 1.68 (dt, $J_{2ax,3}$ = 11.8 Hz, 1 H, *H*-2ax); ^{13}C NMR (D_2O) δ 175.80 (*C*OCH_3′), 174.34 (C-1′), 101.63 (C-1), 100.92 (C-2′), 75.67 (C-5), 72.62 (C-8′), 72.58 (C-6′), 69.13 (C-7′), 69.12 (C-4′), 68.48 (C-6), 68.19 (C-3), 67.21 (C-4), 63.48 (C-9′), 56.88 (OCH_3), 52.66 (C-5′), 40.84 (C-3′), 33.69 (C-2), 22.88 (CO*C*H_3′). Anal. Calcd for $C_{18}H_{31}NO_{13}$·1H_2O: C, 44.34; H, 6.83; N, 2.87. Found: C, 44.97; H, 6.75; N, 2.93.

Methyl (5-Acetamido-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosylonic acid)-(2−3)-2-deoxy-β-D-*lyxo*-hexopyranoside (25). Compounds **1** and **17** were incubated for 24 (*S. typhimurium* sialidase) and 16 h (Newcastle disease virus sialidase) following the respective transsialylation procedures to give 4.7 mg (20%, *S. typhimurium* sialidase) and 3.3 mg (14%, Newcastle disease virus sialidase) of compound **25**, respectively: $[\alpha]^{20}_D$ −19° (*c* 1, H_2O); ^{1}H NMR (D_2O) δ 4.56 (dd, $J_{1,2eq}$ = 2.4 Hz, $J_{1,2ax}$ = 10.0 Hz, 1 H, *H*-1), 4.09 (dd, $J_{3,4}$ = 3.6 Hz, 1 H, *H*-3), 3.93 (bd, $J_{4,5}$ = 1.0 Hz, 1 H, *H*-4), 3.89−3.86 (m, 2 H, *H*-8′, *H*-9′), 3.83 (dd, $J_{5',6'}$ = 10.4 Hz, 1 H, *H*-5′), 3.80−3.76 (m, 2 H, *H*-6a, *H*-6b), 3.71 (ddd, $J_{5,6a}$ = 8.0 Hz, $J_{5,6b}$ = 4.6 Hz, *H*-5), 3.69 (dd, $J_{4',5'}$ = 9.6 Hz, 1 H, *H*-4′), 3.67 (dd, $J_{6',7'}$ = 1.9 Hz, 1 H, *H*-6′), 3.65 (dd, $J_{8',9b'}$ = 6.7 Hz, $J_{9a',9b'}$ = −12.2 Hz, 1 H, *H*-9b′), 3.60 (dd, $J_{7',8'}$ = 9.0 Hz, *H*-7′), 3.54 (s, 3 H, OCH_3), 2.76 (dd, $J_{3eq',4'}$ = 4.6 Hz, $J_{3ax',3eq'}$ = 12.5 Hz, 1 H, *H*-3eq′), 2.00 (ddd, $J_{2eq,3}$ = 5.3 Hz, $J_{2eq,2ax}$ = 12.4 Hz, 1 H, *H*-2eq), 1.80 (t, $J_{3ax',4'}$ = 11.6 Hz, 1 H, *H*-3ax′), 1.68 (dt, $J_{2ax,3}$ = 12.0 Hz, 1 H, *H*-2ax); ^{13}C NMR (D_2O) δ 175.81 (*C*OCH_3′), 174.78 (C-1′), 101.57 (C-1), 100.32 (C-2′), 75.69 (C-5), 75.21 (C-3), 73.54 (C-6′), 72.57 (C-8′), 69.12 (C-4′), 68.87 (C-7′), 67.34 (C-4), 63.47 (C-9′), 61.78 (C-6), 56.85 (OCH_3), 52.50 (C-5′), 40.44 (C-3′), 33.72 (C-2), 22.83 (CO*C*H_3′). Anal. Calcd for $C_{18}H_{31}NO_{13}$: C, 46.05; H, 6.66; N, 2.98. Found: C, 45.67; H, 6.77; N, 2.92.

***O*-(5-Acetamido-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosylonic acid)-(2−6)-*O*-(2-acetamido-2-deoxy-α-D-galactopyranosyl)-(1−3)-L-threonine (26).** Compounds **1** and **2** were incubated for 20 (*V. cholerae* sialidase) and 36 h (*C. perfringens* sialidase) following the respective transsialylation procedures to give 4.9 mg (16%, *V. cholerae* sialidase) and 3.1 mg (10%, *C. perfringens* sialidase) of compound **26**, respectively:. $[\alpha]^{20}_D$ +61° (*c* 0.2, H_2O); ^{1}H NMR (D_2O) δ 4.91 (dd, $J_{1,2}$ = 3.8 Hz, 1 H, *H*-1), 4.37 (dq, $J_{\alpha CH,\beta CH3}$ = 2.4 Hz, 1 H, ThrβCH), 4.16 (dd, $J_{2,3}$ = 11.1 Hz, 1 H, *H*-2), 4.12 (dd, $J_{6a,6b}$ = −10.4 Hz, 1 H, *H*-6a), 4.02 (dd, $J_{4,5}$ = 0.8 Hz, $J_{3,4}$ = 2.7 Hz, 1 H, *H*-4), 3.99 (m, 1 H, *H*-5), 3.90−3.86 (m, 3 H, ThrαCH, *H*-8′, *H*-9a′), 3.83 (dd, $J_{5',6'}$ = 10.2 Hz, 1 H, *H*-5′), 3.70 (dd, $J_{6',7'}$ = 1.4 Hz, 1 H, *H*-6′), 3.66−3.62 (m, 3 H, *H*-9b′, *H*-4′, *H*-6b), 3.58 (dd, $J_{7',8'}$ = 8.9 Hz, 1 H, *H*-7′), 2.71 (dd, $J_{3'eq,3'ax}$ = 12.0 Hz, $J_{3'eq,4'}$ = 4.6 Hz, 1 H, *H*-3′eq), 2.06, 2.03 (2s, 6 H, CO*C*H_3), 1.69 (t, $J_{3'ax,4'}$ = 12.2 Hz, 1 H, *H*-3′ax), 1.35 (d, $J_{\beta CH,\gamma CH3}$ = 6.4 Hz, 3 H, Thr$\gamma$$CH_3$); ^{13}C NMR ($D_2O$) δ 175.77 (*C*OCH_3′), 174.91 (*C*OCH_3), 174.26 (C-1′), 173.84 (Thr*C*O), 101.03 (C-2′), 99.58 (C-1), 76.03 (Thrβ*C*H), 73.23 (C-6′), 72.60 (C-8′), 71.92 (C-5), 69.17 (C-7′), 69.04 (C-4′), 68.94 (C-4), 68.49 (C-3), 64.54 (C-6), 63.48 (C-9′), 59.89 (Thrα*C*H), 52.67 (C-5′), 50.33 (C-2), 40.87 (C-3′), 22.87 (CO*C*H_3′), 22.74 (CO*C*H_3), 19.01 (Thrγ*C*H_3). Anal. Calcd for $C_{23}H_{39}N_3O_{16}$·2H_2O: C, 42.51; H, 6.67; N, 6.47. Found: C, 42.86; H, 6.56; N, 6.51.

***O*-(5-Acetamido-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosylonic acid)-(2−3)-*O*-(2-acetamido-2-deoxy-α-D-galactopyranosyl)-(1−3)-L-threonine (27).** Compounds

1 and **2** were incubated for 24 (*S. typhimurium* sialidase) and 20 h (Newcastle disease virus sialidase) following the respective transsialylation procedures to give 4.6 mg (15%, *S. typhimurium* sialidase) and 3.1 mg (10%, Newcastle disease virus sialidase) of compound **27**, respectively: $[\alpha]^{20}_D$ +69° (*c* 0.4, H_2O); ^{1}H NMR (D_2O) δ 4.94 (d, $J_{1,2}$ = 3.6 Hz, 1 H, *H*-1), 4.42 (dq, $J_{\alpha CH,\beta CH}$ = 2.6 Hz, 1 H, ThrβCH), 4.27 (dd, $J_{2,3}$ = 11.2 Hz, *H*-2), 4.17 (dd, $J_{4,5}$ = 0.8 Hz, H-4), 4.07 (m, 1H, *H*-5), 4.02 (dd, $J_{3,4}$ = 2.9 Hz, 1 H, *H*-3), 3.90−3.87 (m, 3 H, H-8′, H-9a′, ThrαCH), 3.86 (dd, $J_{5',6'}$ = 10.0 Hz, *H*-5′), 3.72 (dd, $J_{4',5'}$ = 9.6 Hz, *H*-4′), 3.69−3.65 (m, 4 H, *H*-6a, *H*-6b, H-6′, H-9b′), 3.59 (dd, $J_{7',8'}$ = 9.4 Hz, *H*-7′), 2.77 (dd, $J_{3eq',4'}$ = 4.5 Hz, $J_{3ax',3eq'}$ = 12.2 Hz, 1 H, *H*-3eq′), 2.05, 2.02, (2s, 6H, $COCH_3$), 1.79 (t, $J_{3ax',4'}$ = 11.2 Hz, 1 H, *H*-3ax′), 1.38 (d, 3 H, $J_{\beta CH,\gamma CH3}$ = 6.6 Hz, ThrγCH_3); ^{13}C NMR (D_2O) δ 175.72 ($COCH_3$′), 175.59 ($COCH_3$), 174.66 (C-1′), 173.77 (Thr*C*O), 100.43 (C-2′), 99.47 (C-1), 78.02 (C-3), 76.37 (Thrβ*C*H), 73.53 (C-6′), 72.63 (C-8′), 71.56 (C-5), 69.37 (C-4), 69.17 (C-4′), 68.83 (C-7′), 63.24 (C-9′), 62.12 (C-6), 59.69 (Thrα*C*H), 52.44 (C-5′), 49.34 (C-2), 40.55 (C-3′), 23.17 ($COCH_3$), 22.77 ($COCH_3$′), 19.52 (ThrγCH_3). Anal. Calcd for $C_{23}H_{39}N_3O_{16}$: C, 45.02; H, 6.41; N, 6.85. Found: C, 44.38; H, 6.58; N, 6.69.

Allyl (5-Acetamido-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosylonic acid)-(2−6) -(*β*-D-galactopyranosyl)-(1−3)-2-azido-2-deoxy-*β*-D-galactopyranoside (9). Compounds **1** and **8** were incubated for 20 (*V. cholerae* sialidase) and 42 h (*C. perfringens* sialidase) following the respective transsialylation procedures to give 4.1 mg (12%, *V. cholerae* sialidase) and 2.4 mg (7%, *C. perfringens* sialidase) of compound **9**, respectively: $[\alpha]^{20}_D$ −51° (*c* 0.9, H_2O); ^{1}H NMR (D_2O) δ 5.96 (m, 1 H, OCH_2CHCH_2), 5.34−5.27 (m, 2 H, OCH_2CHCH_2), 4.51 (d, $J_{1,2}$ = 7.8 Hz, 1 H, H-1), 4.46 (d, $J_{1',2'}$ = 8.0 Hz, 1 H, H-1′), 4.23 (dd, $J_{2,3}$ = 10.2 Hz, $J_{3,4}$ = 3.2 Hz, 1 H, *H*-3), 4.21−4.15 (m, 2 H, OCH_2CHCH_2), 4.12 (dd, $J_{4,5}$ = 1.2 Hz, 1 H, *H*-4), 4.00 (dd, $J_{6a',6b'}$ = −10.2 Hz, 1 H, *H*-6a′), 3.93 (bd, $J_{3',4'}$ = 3.8 Hz, $J_{4',5'}$ = 1.6 Hz, 1 H, *H*-4′), 3.90 (dd, 1 H, H-8″), 3.87 (dd, 1 H, *H*-9a″), 3.83 (dd, $J_{5'',6''}$ = 10.3 Hz, 1 H, *H*-5″), 3.81 (m, 1 H, *H*-5′), 3.75−3.67 (m, 3 H, *H*-6″, *H*-6a, *H*-6b), 3.67 (dd, 1 H, *H*-3′), 3.65 (dd, 1 H, *H*-9b″), 3.61 (dd, $J_{4'',5''}$ = 8.9 Hz, 1 H, *H*-4″), 3.59−3.48 (m, 5 H, *H*-7″, *H*-2′, *H*-6b′, *H*-2, *H*-5), 2.71 (dd, $J_{3eq'',4''}$ = 4.8 Hz, 1 H, *H*-3eq″), 2.03 (s, 3 H, N*H*COCH$_3$), 1.74 (t, $J_{3ax'',4''}$ = 11.9 Hz, $J_{3ax'',3eq''}$ = −12.4 Hz, 1 H, *H*-3ax″); ^{13}C NMR (D_2O) δ 175.82 ($COCH_3$″), 174.28 (C-1″), 133.39 (OCH_2CHCH_2), 119.07 (OCH_2CHCH_2), 104.43 (C-1′), 100.96 (C-2″), 100.78 (C-1), 80.22 (C-3), 75.07 (C-5), 74.44 (C-5′), 73.39 (C-6″), 73.34 (C-3′), 72.57 (C-8″), 71.76 (C-2′), 70.89 (OCH_2CHCH_2), 69.31 (C-7″), 69.24 (C-4′), 69.19 (C-4″), 68.15 (C-4), 64.22 (C-6′), 63.48 (C-9″), 62.84 (C-2), 61.07 (C-6), 52.73 (C-5″), 40.97 (C-3″), 22.88 (CH_3″). Anal. Calcd for $C_{26}H_{42}N_4O_{18}$: C, 44.70; H, 6.06; N, 8.02. Found: C, 45.10; H, 5.94; N, 8.16.

Allyl (5-Acetamido-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosylonic acid)-(2−3)-(*β*-D-galactopyranosyl)-(1−3)-2-azido-2-deoxy-*β*-D-galactopyranoside (10). Compounds **1** and **8** were incubated for 24 (*S. typhimurium* sialidase) and 20 h (Newcastle disease virus sialidase) following the respective transsialylation procedures to give 3.8 mg (11%, *S. typhimurium* sialidase) and 3.9 mg (11%, Newcastle disease virus sialidase) of compound **10**, respectively: $[\alpha]^{20}_D$ −34° (*c* 0.8, H_2O); ^{1}H NMR (D_2O) δ 5.92 (m, 1 H, OCH_2CHCH_2), 5.32−5.24 (m, 2 H, OCH_2CHCH_2), 4.56 (d, $J_{1',2'}$ = 7.8 Hz, 1H, H-1′), 4.53 (d, $J_{1,2}$ = 8.0 Hz, 1 H, H-1), 4.25 (dd, $J_{3,4}$ = 3.6 Hz, 1 H, *H*-3), 4.22−4.17 (m, 2 H, OCH_2CHCH_2), 4.15 (dd, $J_{4,5}$ = 1.5 Hz, 1 H, *H*-4), 4.12−4.08 (m, 2 H, H-3′ and H-4), 3.95 (bd, $J_{4',5'}$ = 0.7 Hz, 1 H, *H*-4′), 3.89 (dd, $J_{8'',9a''}$ = 2.6 Hz, 1 H, *H*-8″), 3.87 (dd, $J_{9a'',9b''}$ = 12.2 Hz, 1 H, *H*-9a″), 3.83 (dd, $J_{5'',6''}$ = 10.2 Hz, 1 H, *H*-5″), 3.71 (m, 2 H, *H*-6a′, *H*-6b′), 3.76−3.63 (m, 6 H, *H*-5′, *H*-4″, *H*-6″, *H*-9b″, *H*-6a, *H*-6b), 3.63 (dd, $J_{2',3'}$ = 9.6 Hz, 1 H, *H*-2′), 3.60 (dd, $J_{7'',8''}$ = 9.6 Hz, 1 H, *H*-7″), 3.57 (dd, $J_{2,3}$ = 10.0 Hz, 1 H, *H*-2), 2.76 (dd, $J_{3eq'',4''}$ = 4.8 Hz, $J_{3ax'',3eq''}$ = −12.2 Hz, 1 H, *H*-3eq″), 2.03 (s, 3 H, N*H*COCH$_3$), 1.80 (t, $J_{3ax'',4''}$ = 12.0 Hz, 1 H, *H*-3ax″); ^{13}C NMR (D_2O) δ 175.84 ($COCH_3$″), 174.64 (C-1″), 133.41 (OCH_2CHCH_2), 119.10 (OCH_2CHCH_2), 105.21 (C-1′), 100.93 (C-1), 100.46 (C-2″), 80.37 (C-3), 76.49 (C-3′), 75.69 (C-5′), 75.15 (C-5), 73.69 (C-6″), 72.62 (C-8″), 70.93 (OCH_2CHCH_2), 69.94 (C-2′), 69.17 (C-4″), 69.01 (C-7″), 68.23 (C-4′), 68.19 (C-4), 63.43 (C-9″), 62.90 (C-2), 61.64 (C-6′), 61.15 (C-6), 52.53 (C-5″), 40.59 (C-3″), 22.83 (CH_3″). Anal. Calcd for $C_{26}H_{42}N_4O_{18}$•$1H_2O$: C, 43.56; H, 6.19; N, 7.82. Found: C, 43.59; H, 6.02; N, 7.87.

***O*-(5-Acetamido-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosylonic acid)-(2−6)-*O*-(*β*-D-galactopyranosyl)-(1−3)-2-acetamido-2-deoxy-α-D-galactopyranosyl)-(1−3)-L-threonine (5).** Compounds **1** and **4** were incubated for 26 (*V. cholerae* sialidase) and 48 h (*C. perfringens* sialidase) following the respective transsialylation procedures to give 4.7 mg (12%, *V. cholerae* sialidase) and 1.6 mg (4%, *C. perfringens* sialidase) of compound **5**, respectively: $[\alpha]^{20}_D$ +7° (*c* 0.6, H_2O); ^{1}H NMR (D_2O) δ 4.91 (d, $J_{1,2}$ = 3.6 Hz, 1 H, *H*-1), 4.46 (d, $J_{1',2'}$ = 7.6 Hz, 1 H, *H*-1′), 4.41 (dq, $J_{\alpha CH,\beta CH}$ = 2.6 Hz, 1 H, ThrβCH), 4.27 (dd, $J_{2,3}$ = 11.2 Hz, 1 H, *H*-2), 4.21 (dd, $J_{4,5}$ = 0.8 Hz, 1 H, *H*-4), 4.07 (dd, $J_{3,4}$ = 2.8 Hz, 1 H, *H*-3), 4.05 (m, 1 H, *H*-5), 3.98 (dd, $J_{6a',6b'}$ = −10.2 Hz, 1 H, H-6a′), 3.93−3.83 (m, 4 H, *H*-4′, *H*-8″, *H*-9a″, ThrαCH), 3.82−3.80 (m, 2 H, *H*-5′, *H*-5″), 3.70 (dd, $J_{6'',7''}$ = 1.7 Hz, 1 H, *H*-6″), 3.69−3.61 (m, 5 H, *H*-3′, *H*-4″, *H*-9b″, *H*-6a, *H*-6b), 3.56 (dd, $J_{7'',8''}$ = 8.4 Hz, 1 H, *H*-7″), 3.54 (dd, $J_{2',3'}$ = 9.8 Hz, 1 H, *H*-2′), 3.52 (dd, 1 H, *H*-6b′), 3.50 (dd, $J_{2',3'}$ = 9.8 Hz, 1 H, *H*-2′), 2.72 (dd, $J_{3eq'',4''}$ = 4.6 Hz, $J_{3ax'',3eq''}$ = 12.2 Hz, 1 H, *H*-3eq″), 2.02, 2.01 (2s, 6 H, $COCH_3$), 1.67 (t, $J_{3ax'',4''}$ = 12.0 Hz, 1 H, *H*-3ax′), 1.35 (d, $J_{\beta CH,\gamma CH}$ = 6.6 Hz, 3 H, ThrγCH); ^{13}C NMR (D_2O) δ 175.82 ($COCH_3$″), 175.00 ($COCH_3$′) 174.31 (C-1″), 173.64 (Thr*C*O), 105.87 (C-1′), 100.79 (C-2″), 99.62 (C-1), 78.02 (C-3), 76.01 (Thrβ*C*H), 75.62 (C-3′, C-5′), 73.40 (C-6″), 72.54 (C-8″), 71.92 (C-2′), 71.62 (C-5), 69.82 (C-4′), 69.49 (C-4), 69.22 (C-7″), 69.13 (C-4″), 64.53 (C-6′), 63.53 (C-9″), 61.74 (C-6), 59.67 (Thrα*C*H), 52.74 (C-5″), 48.93 (C-2), 40.79 (C-3″), 23.24 ($COCH_3$), 22.88 ($COCH_3$″), 19.32 (ThrγCH_3). Anal.Calcd for $C_{29}H_{49}N_3O_{21}$•$2H_2O$: C, 42.89; H, 6.58; N, 5.18. Found: C, 43.13; H, 6.46; N, 5.32.

***O*-(5-Acetamido-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosylonic acid)-(2−3)-*O*-(*β*-D-galactopyranosyl)-(1−3)-2-acetamido-2-deoxy-α-D-galactopyranosyl)-(1−3)-L-threonine (6).** Compounds **1** and **4** were incubated for 24 (*S. typhimurium* sialidase) and 20 h (Newcastle disease virus sialidase) following the respective transsialylation procedures to give 4.3 mg (11%, *S. typhimurium* sialidase) and 3.9 mg (10%, Newcastle disease virus sialidase) of compound **6**, respectively. Analytical data were identical to published results.[28]

Methyl (5-Acetamido-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosylonic acid)-(2−6)-(*β*-D-galactopyranosyl)-(1−4)-2-deoxy-α-D-*lyxo*-hexopyranoside (13). Compounds **1** and **12** were incubated for 22 (*V. cholerae* sialidase) and 28 h (*C. perfringens* sialidase) following the respective transsialylation procedures to give 4.7 mg (15%, *V. cholerae* sialidase) and 1.6 mg (5%, *C. perfringens* sialidase) of compound **13**, respectively: $[\alpha]^{20}_D$ +14° (*c* 1, H_2O); ^{1}H NMR (D_2O) δ 5.09 (dd, $J_{1,2eq}$ = 1.0 Hz, $J_{1,2ax}$ = 3.8 Hz, 1 H, *H*-1), 4.44 ($J_{1',2'}$ = 8.0 Hz, 1 H, *H*-1′), 4.14 (ddd, $J_{3,4}$ = 3.1 Hz, 1 H, *H*-3), 4.07 (bd, $J_{4,5}$ = 0.9 Hz, 1 H, *H*-4), 3.97 (dd, 1 H, $J_{6a',6b'}$ = −10.2 Hz, *H*-6a′), 3.93 (m, 1 H, *H*-4′), 3.88−3.85 (m, 3 H, *H*-5, *H*-9a″, *H*-8″), 3.84−3.82 (m, 2 H, *H*-5′, *H*-5″), 3.73 (m, 2 H, *H*-6a, *H*-6b), 3.68−3.63 (m, 4 H, *H*-4″, *H*-3′, *H*-9a″, *H*-6″), 3.56−3.54 (m, 3 H, *H*-2′, *H*-6b′, *H*-7″), 3.54 (s, 3 H, OCH_3), 3.52 (m, $J_{2',3'}$ = 9.9 Hz, 1 H, *H*-2′), 2.68 (dd, $J_{3eq'',4''}$ = 4.8 Hz, $J_{3eq'',3ax''}$ = 12.3 Hz, 1 H, *H*-3ax″), 1.99−1.95 (m, 2 H, *H*-2ax, *H*-2eq), 1.72 (t, $J_{3ax'',4''}$ = 12.2 Hz, 1 H, *H*-3ax″); ^{13}C NMR (D_2O) δ 175.81 ($COCH_3$), 174.35 (C-1″), 104.27 (C-1′), 100.89 (C-2″), 98.69 (C-1), 75.81 (C-4), 74.53 (C-5′), 73.44 (C-6″), 73.19 (C-3′), 72.61 (C-8″), 71.59 (C-2′), 71.24 (C-5), 69.31 (C-7″), 69.22 (C-4′), 69.08 (C-4″), 68.16 (C-3), 67.88 (C-6), 64.18 (C-6′), 63.53 (C-9″), 54.80 (OCH_3), 52.71 (C-5″), 40.86 (C-3″), 31.52 (C-2), 22.91 ($COCH_3$). Anal. Calcd for $C_{24}H_{41}NO_{18}$: C, 45.64; H, 6.54; N, 2.22. Found: C, 45.72; H, 6.62; N, 2.19.

Methyl (5-Acetamido-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosylonic acid)-(2−3)-(*β*-D-galactopyrano-

(28) Gambert, U.; Thiem, J. *Eur. J. Org. Chem.* **1999**, 107−110.
(29) Bergh, M. L. E.; Koppen, P.; van den Eijnden, D. H. *Carbohydr. Res.* **1981**, *94*, 225−229.

syl)-(1−4)-2-deoxy-α-D-*lyxo*-hexopyranoside (14). Compounds **1** and **12** were incubated for 22 (*S. typhimurium* sialidase) and 20 h (Newcastle disease virus sialidase) following the respective transsialylation procedures to give 3.2 mg (10%, *S. typhimurium* sialidase) and 2.5 mg (8%, Newcastle disease virus sialidase) of compound **14**, respectively: $[\alpha]^{20}_{D}$ +25° (*c* 1, H_2O); ^{1}H NMR (D_2O) δ 5.06 (dd, $J_{1,2eq}$ = 1.0 Hz, $J_{1,2ax}$ = 3.6 Hz, 1 H, *H*-1), 4.56 (d, $J_{1',2'}$ = 8.0 Hz, 1 H, *H*-1′), 4.16 (ddd, $J_{3,4}$ = 3.2 Hz, 1 H, *H*-3), 4.12 (bd, bd, $J_{4,5}$ = 0.8 Hz, 1 H, *H*-4), 4.09 (dd, $J_{3',4'}$ = 3.6 Hz, 1 H, *H*-3′), 3.94 (bd, $J_{4',5'}$ = 0.8 Hz, 1 H, *H*-4′), 3.91 (ddd, $J_{5,6a}$ = 6.3 Hz, $J_{5,6b}$ = 6.4 Hz, 1 H, *H*-5), 3.88−3.85 (m, 2 H, *H*-8″, *H*-9b″), 3.84 (dd, $J_{5'',6''}$ = 10.4 Hz, 1 H, *H*-5″), 3.73−3.64 (m, 6 H, *H*-4″, *H*-5′, *H*-6a′, *H*-6b′, *H*-6a, *H*-6b), 3.66 (dd, $J_{6'',7''}$ = 1.8 Hz, 1 H, *H*-6″), 3.65 (m, 1 H, *H*-9b″), 3.61 (dd, $J_{7'',8''}$ = 9.6 Hz, 1 H, *H*-7″), 3.57 (s, 3 H, OCH_3), 3.52 (m, $J_{2',3'}$ = 9.8 Hz, 1 H, *H*-2′), 2.76 (dd, 1 H, $J_{3eq'',4''}$ = 4.8 Hz, $J_{3eq'',3ax''}$ = −12.4 Hz, *H*-3eq″), 2.01−1.99 (m, 2 H, *H*-2ax, *H*-2eq), 1.80 (t, 1 H, $J_{3ax'',4''}$ = 12.2 Hz, *H*-3ax″); ^{13}C NMR (D_2O) δ 175.80 ($COCH_3$″), 174.71 (C-1″), 104.38 (C-1′), 100.43 (C-2″), 98.74 (C-1), 76.43 (C-3′), 75.89 (C-5′), 75.72 (C-4), 73.67 (C-6″), 72.61 (C-8″), 71.22 (C-5), 70.08 (C-2′), 69.18 (C-4″), 68.94 (C-7″), 68.15 (C-4′), 68.08 (C-3), 67.86 (C-6), 63.38 (C-9″), 61.94 (C-6′), 54.77 (OCH_3), 52.54 (C-5″), 40.72 (C-3″), 31.44 (C-2), 22.87 ($COCH_3$″). Anal. Calcd for $C_{24}H_{41}NO_{18}$: C, 45.64; H, 6.54; N, 2.22. Found: C, 45.44; H, 6.61; N, 2.25.

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Supporting Information Available: ^{13}C NMR spectra of the compunds **5**, **9**, **10**, **12**−**14**, and **22**−**27**. This material is available free of charge via the Internet at http://pubs.acs.org.

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