8-O-Sialylation of Neuraminic Acid

Julio C. Castro-Palomino, Yury E. Tsvetkov, and Richard R. Schmidt*

Contribution from the Fakultät Chemie, Universität Konstanz, Fach M 725, D-78457 Konstanz, Germany

Received November 26, 1997

Abstract: Synthesis of Neu5Acα(2−3)Galβ(1−4)Glc and Neu5Acα(2−8)Neu5Ac derivatives **12**α and **14**α, from lactose derivative **6** and 2,3-dehydro-Neu5Ac derivative **7**, respectively, with anchimerically assisted Neu5Ac donor **3e**, **3f**, or **3g** has been studied. Reaction of halogenose **3e**, having a 3-phenoxythiocarbonyloxy moiety as the assisting group, afforded with **6** and **7** in the presence of equimolar amounts of AgOTf as promoter α-sialosides **11**α and **13**α which were readily deoxygenated to afford **12**α and **14**α, respectively. Reaction of phosphite **3f**, having a 3-bromo substituent as the potentially anchimeric assisting group, furnished in the presence of catalytic amounts of TMSOTf with **6** as acceptor α-sialoside **15**α and with **7** as acceptor β-sialoside **17**β, which gave on debromination **12**α and **14**β, respectively. Reaction of readily available phosphite **3g**, having a 3-thiobenzoyloxy group as the anchimeric assisting group, afforded with **6** and **7** in the presence of catalytic amounts of TMSOTf in very high yields α-linked sialosides **21**α and **22**α, which could be readily deoxygenated to afford **12**α and **14**α. Thus, **3g** is an almost ideal sialyl donor for the high-yielding generation of α-glycosidic linkages also to low-reactive acceptors.

Introduction

The sequences Neu5Acα(2−3)Gal and Neu5Acα(2−8)-Neu5Ac and higher homooligomers thereof are frequently occurring constituents of glycoconjugates including various gangliosides. The derived compounds were found to play important roles in numerous biological phenomena, being for instance tumor-associated antigens[1,2] or receptors of bacterial toxins and viruses.[2,3]

Several successful approaches to the generation of the Neu5Acα(2−3)Gal linkage have been reported.[4,5] High α-selectivity and good yields have been gained with alkyl/arylthio leaving groups, which require equimolar or higher amounts of a thiophilic promoter system,[4,6] or with phosphites as leaving groups, which require just catalytic amounts of an acid for activation; especially favorable results were obtained when the reaction was carried out in acetonitrile at low temperatures,[4,7,8] because, as demonstrated by us, the "nitrile effect" can greatly influence anomeric stereocontrol.[7,9] However, these methods failed in the generation of the highly demanding Neu5Acα(2−8)Neu5Ac linkage.[10,11]

To solve the problem of α(2−8)-linkage, anchimeric assistance by an auxiliary group at C-3 has been proposed.[12−15] Thus, addition of X′Y′ reagents to 2,3-dehydroneuraminic acid **1** (Scheme 1), affording **2**α and **2**β, and then transformation into desired **3** with Y on the β-side was performed, where Y represents a group prone to neighboring group participation. Mainly HOBr and PhSCl addition to **1** (R = Ac, Bn) were investigated (see Table 1). HOBr addition did not only lead to the wrong regioisomers (because a 3-oxygen atom was envisaged for the anchimeric assistance), but also α- and β-face addition (→**2a**α,β and **2b**α,β) was obtained; then, in a quite tedious procedure, both compounds were transformed into desired **3a** and **3b**, respectively (entries 1 and 2).[12] PhSCl addition gave the desired regioisomers (a 3-sulfur atom was considered for the anchimeric assistance), yet again, α- and β-face addition was observed (entries 3 and 4; **2c**α,β); therefore, chromatographic separation of **2c**α and **2c**β and transformation into **3c** were required (entry 3).[13,14] Then, with the help of promoter systems, from **3a**−**3c** eventually intermediate **4** was generated which should result in α(2−8)-sialoside formation. Table 1 exhibits that for most cases studied excellent α-selectivities could be obtained, however, the overall yields are not yet satisfactory. Only when a more reactive, yet not readily available, *O*-benzyl-protected neuraminic acid derivative was employed as sialyl donor (entry 2) acceptable product yields were found.[13] With the 3-hydroxy group (entry 1), which is less prone to anchimeric assistance than the 3-phenylthio moiety, also the β(2−8)-sialoside was obtained;[12] this is presumably

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(2) Reisfeld, R. A.; Cheresh, D. A. *Adv. Immunl.* **1987**, *40*, 323−377.
(3) Karlsson, K. A. *Annu. Rev. Biochem.* **1989**, *58*, 309−350.
(4) Martin, T. J.; Brescello, R.; Toepfer, A.; Schmidt, R. R. *Glycoconjugate J.* **1993**, *10*, 16−25 and refs 1−37 and 42−44 therein.
(5) Castro-Palomino, J. C.; Ritter, G.; Fortunato, S.; Reinhardt, S.; Old, L. J.; Schmidt, R. R. *Angew. Chem.* **1997**, *109*, 2081−2085; *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 1998−2001.
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(7) Schmidt, R. R. Lecture at Fuji 1990 Symposium, August 1990: partly published in *Carbohydrates-Synthetic Methods and Application in Medicinal Chemistry*; Ogura, H., Hasegawa, A., Suami, T., Eds.; Kodanasha: Tokyo, 1990; pp 66−88.
(8) Martin, T. J.; Schmidt, R. R. *Tetrahedron Lett.* **1992**, *33*, 6123−6126.
(9) Schmidt, R. R.; Rücker, E. *Tetrahedron Lett.* **1980**, *21*, 1421−1424; Schmidt, R. R.; Michel, J. *J. Carbohydr. Chem.* **1985**, *4*, 141−169. Schmidt, R. R.; Behrendt, M. N.; Toepfer, A. *Synlett* **1990**, 694−696.
(10) (a) Castro-Palomino, J. C.; Tsvetkov, Y. E.; Schneider, R.; Schmidt, R. R. *Tetrahedron Lett.* **1997**, *38*. (b) Tsvetkov, Y. E.; Schmidt, R. R. *Tetrahedron Lett.* **1994**, *35*, 8583−8586; *Carbohydr. Lett.* **1996**, *2*, 149−156.
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(12) Okamoto, K.; Kondo, T.; Goto, T. *Tetrahedron* **1988**, *44*, 1291−1298.
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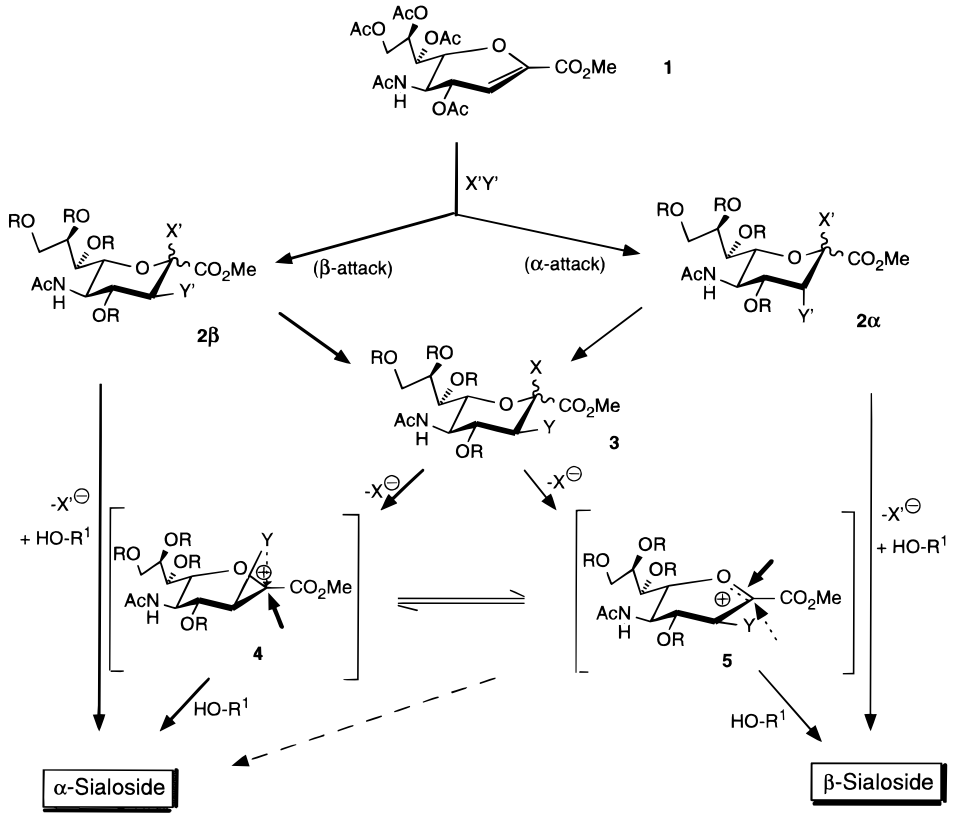
S0002-7863(97)04032-8 CCC: $15.00
Published on Web 05/23/1998

Scheme 1[a]



[a] R = Ac, Bn; HO-R[1] = sialyl acceptor; for details, see Table 1.

Table 1. Anchimerically Assisted Neu5Ac donors Used for the Synthesis of Neu5Acα(2−8)Neu5Ac Disaccharides[a]

	1 → 2α,β					2α,β → **3**					**3** → Neu5Acα(2−8)Neu5Ac disaccharide derivative		
entry	**2**	R	X′	Y′	α:β	**3**	R	X	Y	yield from **1** (%)	promoter (equiv)	yield (%)[h]	α:β
1[b]	**2a**	Ac	OH	Br	3:1	**3a**	Ac	Br	OH	66	AgOTf (1)	26 (17)	3:2
2[c]	**2b**	Bn	OH	Br	4:1	**3b**	Bn	Br	SPh	43[g]	$Hg(CN)_2$ (1.6) $HgBr_2$ (0.5)	64 (28)	α
3[d]	**2c**	Ac	Cl	SPh	1:3	**3c**	Ac	SEt	SPh	52	MSB (1.4) AgOTf (1.4)	28 (14)	>99:1
4[e]	**2c**	Ac	Cl	SPh	1:5[f]	**3d**	Ac	Cl	SPh	49	AgOTf (2)	49 (24)	α

[a] For details, see Scheme 1. [b] Reference 12. [c] Reference 13. [d] Reference 14. [e] Reference 15. [f] This ratio could not be confirmed; see ref 14. [g] Yield contains transformation of **1** into *O*-benzyl-protected derivative. [h] Yield in parentheses: total yield from **1**.

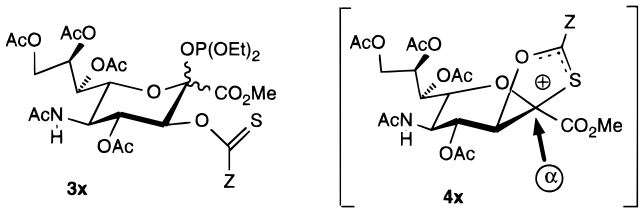
due to the occurrence of transient intermediate **5** which favors via stereoelectronic control β-product formation. These lengthy and not too efficient methodologies for Neu5Acα(2−8)Neu5Ac formation, though not really required, have also been employed for the generation of Neu5Acα(2−3)Gal linkages.[16]

Aims of This Work. To improve the efficiency and simplicity of the Neu5Acα(2−8)Neu5Ac linkage methodology at least five demands should be fulfilled: (i) X′Y′ addition to **1** (Scheme 1) should lead in high yield only to **2**β, thus giving ready access to the decisive sialyl donor **3**; (ii) activation of **3** should be achieved by simple catalysis, as for instance provided by acid treatment of the phosphite leaving group (and not by equal amounts or even more of a promoter system as required for halogen and alkyl/arylthio leaving groups, respectively); (iii) efficient neighboring group participation should be provided in order to enforce highly α-selective reactions also with less reactive acceptors and thus preventing any other side reactions; (iv) the anchimerically assisting group should also accommodate its immediate and practically quantitative removal after product formation; (v) the Neu5Ac donor and/or acceptor should be designed in such a way as to permit repetitive glycosylations, thus making the highly valuable α(2−8)-linked homooligomers of Neu5Ac available and also structural variants thereof.

Obviously, a compound of the general structure **3x** could accommodate demands i−iv: acid-catalyzed removal of the phosphite moiety should lead to relatively stable five-membered oxathiolanium intermediate **4x**[17] which, on proper selection of group Z, will not result in loss of Z^+ or give with the acceptor an ortho ester derivative, but instead it will smoothly lead to the desired α-sialoside linkage in high yield.

(16) (a) Okamoto, K.; Goto, T. *Tetrhadeton* **1990**, *46*, 5835−5857 and references therein. (b) Ito, Y.; Ogawa, T. *Tetrahedron* **1990**, *46*, 89−96. (c) Martichonik, V.; Whitesides, M. G. *J. Am. Chem. Soc.* **1996** , *118*, 8187−8191. (d) Tomoo, T.; Kondo, T.; Abe, H.; Tsukamoto, S.; Isobe, M.; Goto, T. *Carbohydr. Res.* **1996**, *284*, 207−222. (e) Kononoy, L.; Ito, Y.; Ogawa, T. *Tetrahedron Lett.* **1997**, *38*, 1599−1602. (f) Martichonok, V.; Whitesides, M. G. *Carbohydr. Res.* **1997**, *302*, 123−129.

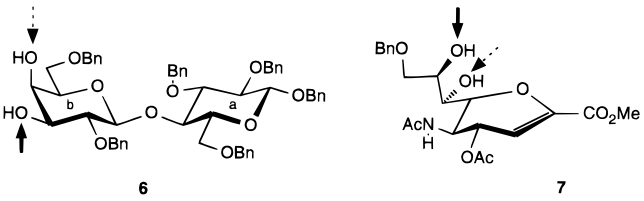
(17) A thiocarbonyloxy moiety was claimed to support 2-deoxy-β-ribonucleoside formation via a six-membered intermediate; yet, still both anomers were obtained: Mukaiyama, T.; Hirano, N.; Nishida, M.; Uchiro, H. *Chem. Lett.* **1996**, 99−100.



To test this concept, we have first investigated the phenoxythiocarbonyloxy group as the anchimerically assisting group and chloride as the leaving group (**3e:** X = Cl; Y = PhO−C(=S)−O).[18] Then we investigated the usefulness of phosphite as the leaving group in the presence of bromine as the anchimericaly assisting group (**3f**: X = $OP(OEt)_2$; Y = Br). Finally the combination of the phosphite leaving group and the thiobenzoyloxy group as the anchimerically assisting group was investigated (**3g**: X = $OP(OEt)_2$; Y = PhC(=S)−O)). In addition, β-face selective addition of the Y′ group (**1** → **2**β) had to be solved.

Results and Discussion

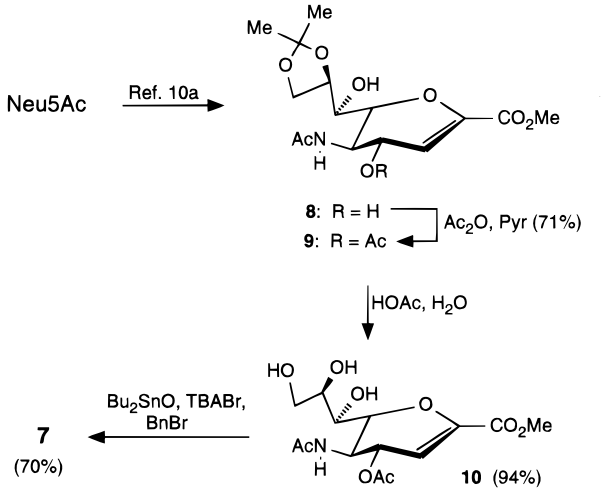
Acceptor Synthesis. The glycosyl donor properties of the newly designed sialyl donors were investigated with two important acceptor molecules. The first one was known 3b,-4b-O-unprotected lactose **6**[19] which exhibited in sialylation reactions with nonanchimerically assisted sialyl donors good yields and mainly α-product formation.[4,5]



As the neuraminic acid derived acceptor, 7,8-O-unprotected 2,3-dehydroneuraminic acid derivative **7** was chosen, because it should combine good reactivity with the potential to reiterate the sialylation procedure (see above, demand v). Compound **7** can be readily prepared from known 8,9-*O*-isopropylidene-2,3-dehydroneuraminic acid derivative **8** (Scheme 2)[10a] which after regioselective 4-O-acetylation with acetic anhydride in pyridine (→**9**) and then acid-catalyzed removal of the *O*-isopropylidene group yielded 7,8,9-O-unprotected derivative **10**. Treatment with dibutyltin oxide (Bu_2SnO) and then benzyl bromide in the presence of tetrabutylammonium bromide (TBABr) led to regioselective 9-O-benzylation, furnishing target molecule **7** in good overall yield.

The Phenoxythiocarbonyl Group as the Anchimerically Assisting Group. Readily available 2,3-dehydroneuraminic acid derivative **1** (Scheme 3) was converted by Goto et al.[12] via HOBr addition, separation from the undesired diastereoisomer, transformation into the 2,3-epoxide, and then treatment with $TiCl_4$ into halogenose **2a**β (see the discussion in the Introduction and Table 1). We found that **2a**β can be directly transformed with phenoxythiocarbonyl chloride in the presence of 4-(dimethylamino)pyridine (DMAP) into *O*-phenoxythiocarbonyl derivative **3e**β in high yield. Reaction of **3e**β with lactose derivative **6** as the acceptor in the presence of equimolar amounts of silver trifluoromethanesulfonate (AgOTf) in 1,2-dichloroethane as solvent afforded the desired α-linkage,

Scheme 2



providing trisaccharide **11**α in high yield. Ensuing treatment with tributyltin hydride (Bu_3SnH)/azoisobutyronitrile (AIBN) in toluene afforded known α(2−3)-linked ganglioside GM3 intermediate **12**α[4] in very high overall yield. Application of the same reaction sequence to 7,8-O-unprotected neuraminic acid derivative **7** as the acceptor afforded exclusively α(2−8)-linked disaccharide **13**α in 68% isolated yield; removal of the *O*-phenylthiocarbonate group led to desired 3-deoxy derivative **14**α in practically quantitative yield. The structural assignment of **14**α could be readily based on the NMR data (^{13}C chemical shifts and $^3J_{C-1',H-3ax'}$) and their comparison with the data of the corresponding β-isomer **14**β which was independently obtained (see below): **14**α, $^3J_{13C(C-1')-1H(H-3ax')} = 7.6$ Hz corresponds to an α-linkage; **14**β, $^3J_{^{13}C(C-1')-1H(H-3ax')} = 1.8$ Hz corresponds to a β-linkage.[20] Thus, it could be demonstrated that the thiocarbonyloxy group is an ideal anchimerically assisting group, leading, presumably via intermediate **4x**, exclusively to α-linkage.

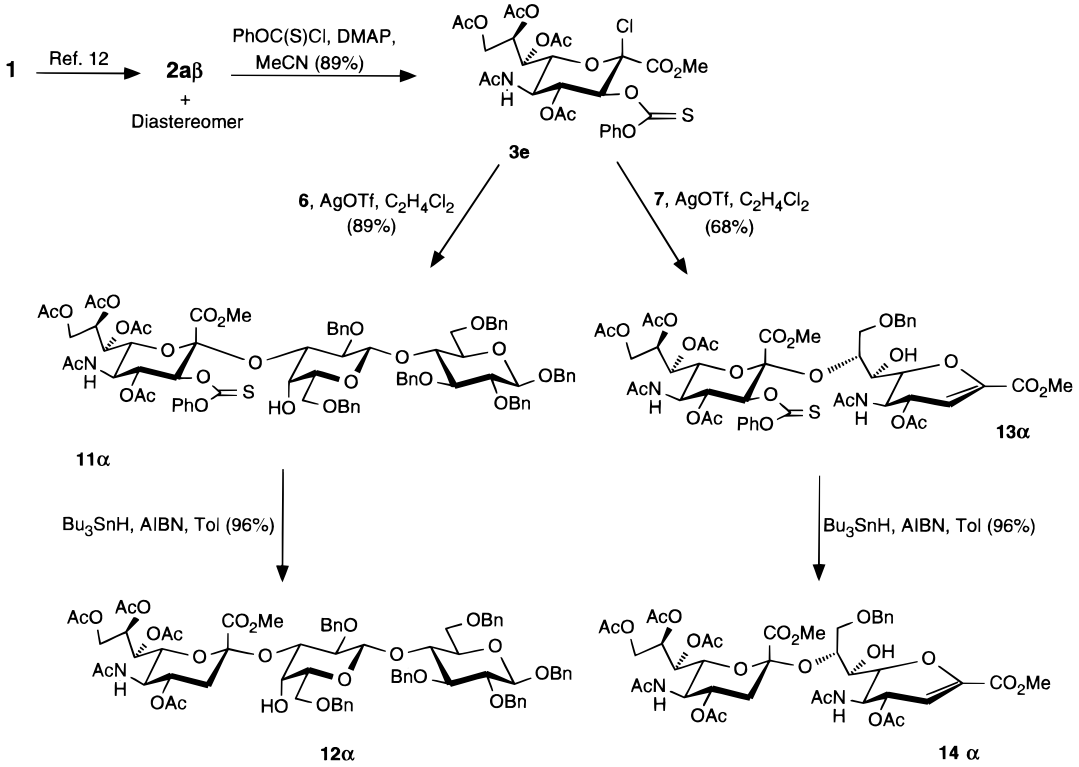
Phosphite as the Leaving Group. For the investigation of the usefulness of phosphite as the leaving group, **1** was transformed into known 3-bromoneuraminic acid derivative **2b**β (Scheme 4)[16a] which had to be separated from the diastereoisomer (see the discussion in the Introduction and Table 1). Reaction of **2b**β with $ClP(OEt)_2$ in the presence of Hünig's base afforded diethyl phosphite derivative **3f** in practically quantitative yield. Because intermediate bromonium ion formation is known to favor anti-addition products, reaction of **3f** with acceptor **6** in the presence of catalytic amounts of trimethylsilyl trifluoromethanesulfonate (TMSOTf; 0.2 equiv) was expected to give α-sialoside. This was confirmed: as the product, lactone **15**α was obtained. Debromination with Bu_3SnH/AIBN in toluene afforded in practically quantitative yield **16**α which upon treatment with NaOMe/MeOH furnished known GM3 intermediate **12**α. Application of the same reaction sequence to acceptor **7** led to a surprising result: presumably via **5** as the intermediate (Scheme 1: R = Ac, Y = Br), only β-linkage to the 8-hydroxy group of **7** was found, thus resulting in β-product **17**β. Debromination of **17**β as described above afforded β-linked disaccharide **14**β, thus permitting unequivocal structural assignments of anomers **14**α and **14**β, respectively. Obviously, phosphite can be successfully employed as the leaving group also with neuraminic acid derivatives having electron-withdrawing substituents at the 3-position. However, C-3 substituents

(18) For preliminary publication of part of this approach, see ref 10a.

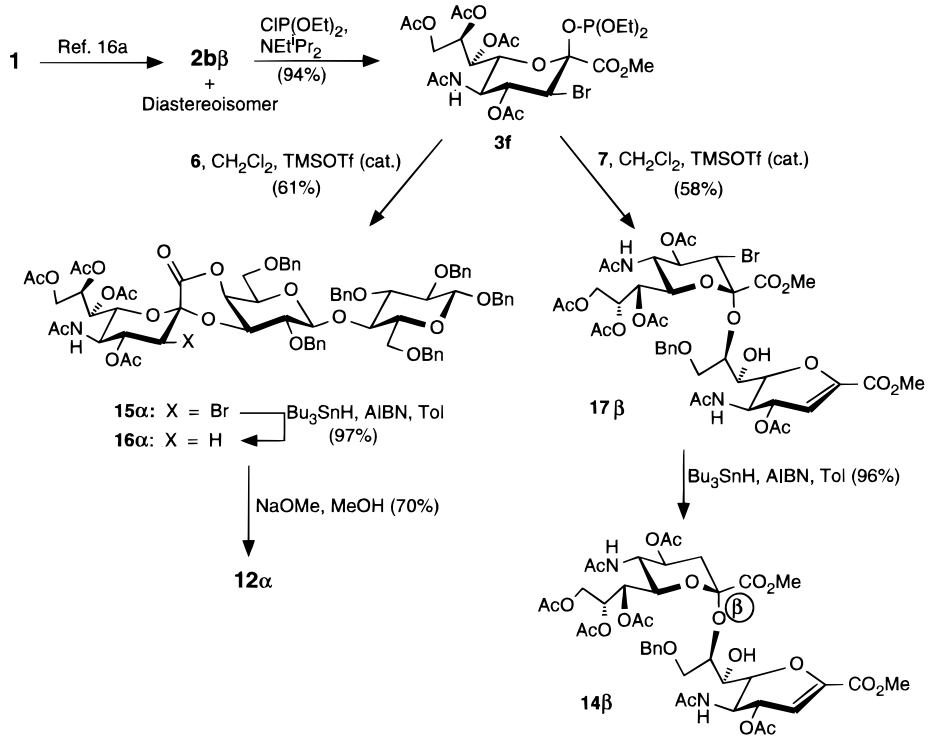
(19) Jung, K.-H.; Hoch, M.; Schmidt, R. R. *Liebigs Ann. Chem.* **1989**, 1099−1106.

(20) Hori, H.; Nakajima, T.; Nishida, Y.; Ohrui, H.; Meguro, H. *Tetrahedron Lett.* **1988**, *29*, 6317−6320.

Scheme 3



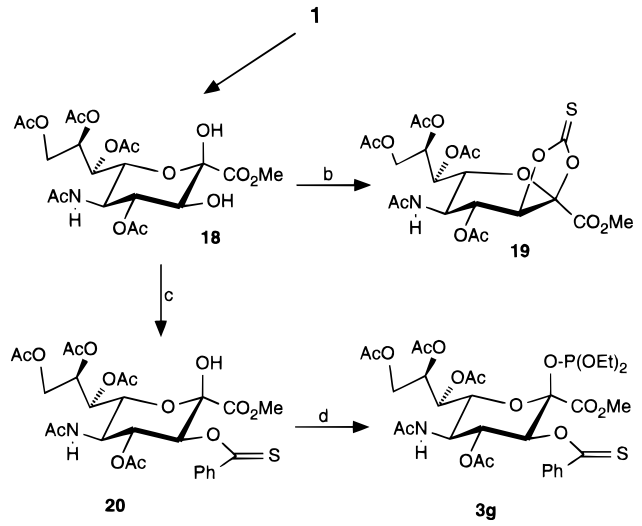
Scheme 4



requiring three membered onium transition state formation in order to exert anchimeric assistance (**4** in Scheme 1) are not as reliable in anomeric control as groups available to five-membered onium intermediate formation, as for instance the thiocarbonyloxy group investigated above (see **4x**).

Phosphite as the Leaving Group and Thiobenzoyloxy as the Anchimerically Assisting Group. For an efficient synthesis of the desired sialyl donor having a phosphite leaving group and thiocarbonyloxy as the anchimerically assisting group at the β-face of C-3, first diastereoselective transformation of **1** into 3-hydroxyneuraminic acid derivative **18** was required (Scheme 5). It turned out that osmium tetroxide catalyzed dihydroxylation using *N*-methylmorpholine *N*-oxide (NMO) as oxidizing agent, following a general procedure,[21] affords via a β-selective dihydroxylation only 2,3-dihydroxy derivative **18** (^{1}H NMR: H-3, $J_{3,4} = 9.5$ Hz), thus readily solving an important demand in our endeavors. Regioselective attachment of a thiocarbonyl group at the 3-hydroxy group with phenoxythio-

Scheme 5[a]



[a] Reagents and conditions: (a) OsO_4/NMNO, acetone, water, *tert*-butyl alcohol (95%); (b) PhOC(S)−Cl, DMAP or PhOC(S)−Cl, Bu_2SnO or PhOC(S)−Cl, $(Bu_3Sn)_2O$ (qu); (c) PhC(Cl)═$NMe_2^+Cl^-$; H_2S (92%); (d) $ClP(OEt)_2$, NEt^iPr_2 (93%).

carbonyl chloride and different bases furnished only cyclic thiocarbonate **19** in practically quantitative yield. Therefore, regioselective introduction of the thiobenzoyl moiety was studied, because this group is not available to cyclization and it can also be readily removed in a radical-initiated deoxygenation reaction.[22] To this aim, **18** was treated with *N*,*N*-dimethyl-α-chlorobenzimidium chloride; ensuing reaction with hydrogen sulfide afforded 3-*O*-thiobenzoyl derivative **20** in very high yield. This compound could be transformed with $ClP(OEt)_2$ in the presence of Hünig's base into desired sialyl donor **3g** in very high overall yield starting from readily accessible **6**.

Reaction of sialyl donor **3g** with acceptor **6** in acetonitrile at −15 °C in the presence of catalytic amounts of TMSOTf (0.2 equiv) afforded α(2−3)-linked trisaccharide **21**α in 88% yield (Scheme 6). Also removal of the anchimerically assisting group could be successfully performed under standard conditions in very high yield, thus affording known GM3 intermediate **12**α. Application of this reaction sequence to **7** as the acceptor led to similar results: exclusively α(2−8)-linkage was observed, affording disaccharide **22**α in 83% isolated yield, which upon removal of the thiobenzoyloxy moiety furnished desired **14**α, yet now in excellent overall yield; thus, the power of sialyl donor **3g** is exhibited, which obviously reacts exclusively via α-face attack of the acceptor at intermediate **4x**.

Conclusion

The demands for an efficient generation of the Neu5Acα-(2−8)Neu5Ac linkage could be well fulfilled. The *β*-selective and practically quantitative dihydroxylation of 2,3-dehydroneuraminic acid derivative **1** affords **18** which can be regioselectively and efficiently transformed into sialyl donor **3g** (overall yield from **1**: 80%). Activation of **3g** with catalytic amounts of TMSOTf (0.2 equiv) provides with 7,8-O-unprotected 2,3-dehydroneuraminic acid derivative **7** exclusively α(2−8)-linkage in very high yield; ensuing convenient removal of the anchimerically assisting group afforded disaccharide **14**α (isolated yield from **3g**, 83%; total yield of **14**α from **1**, 67%). Compound **14**α offers also repetitive application of this methodology. Thus, a convenient and highly efficient methodology for the synthesis of α-sialosides and especially of α-(2−8)-linked neuraminic acids and possibly their homooligomers could be developed.

Experimental Section

General Procedures. Solvents were purified in the usual way. Boiling range of the petroleum ether (PE) used: 35−60 °C. ^{1}H NMR spectra: Bruker AC 250 (250 MHz) and Bruker DRX 600 (600 MHz); solvent, $CDCl_3$; internal standard, Me_4Si; chemical shifts and coupling constants were partially obtained from COSY spectra. Flash chromatography: silica gel (Baker, particle size 40 μm). Thin-layer chromatography (TLC): foil plates, silica gel 60 F_{254} (Merck; layer thickness, 0.1 nm). Optical roations: Perkin-Elmer polarimeter 241 MC; 1 dm cell; temperature, 20 °C. Elemental analyses: Heraeus CHN-O-rapid.

Methyl 5-Acetamido-4-*O*-acetyl-8,9-*O*-isopropylidene-2,3,5-trideoxy-D-*glycero*-D-*galacto*-non-2-enopyranosonate (9). To a solution of known **8**[10a] (1.37 g, 3.97 mmol) in dichloromethane (25 mL) and pyridine (5 mL) was added acetic anhydride (0.94 mL, 9.93 mmol), and the mixture was stirred at room temperature for 15 h. Excess acetic anhydride was destroyed with methanol (1 mL), and the solvents were evaporated with toluene. Column chromatography of the residue (toluene/acetone, 7:3) gave **9** (1.09 g, 71%) as a white powder, which crystallized from a hexanes−ethyl acetate mixture: mp 186−188 °C; $[\alpha]_D$ 61.5° (*c* 1.5, $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.33, 1.38 (s, 3 H, CH_3-isoprop), 2.03, 2.09 (s, 3 H, Ac), 3.46 (dd, 1 H, $J_{6,7}$ = 1.3, $J_{7,8}$ = 8.3 Hz, 7-H), 3.76 (s, 3 H, COOMe), 4.01 (dd, 1 H, $J_{6,7}$ = 1.3, $J_{5,6}$ = 11.2 Hz, 6-H), 4.08 (m, 1 H, 9a-H), 4.14 (m, 1 H, 9b-H), 4.20 (m, 1 H, 5-H), 4.36 (m, 1 H, 8-H), 5.66 (dd, 1 H, $J_{3,4}$ = 2.5, $J_{4,5}$ = 9.2 Hz, 4-H), 5.82 (d, 1 H, $J_{3,4}$ = 2.5 Hz, 3-H), 6.02 (d, 1 H, NH). Anal. Calcd for $C_{17}H_{25}NO_9$: C, 52.71; H, 6.46; N, 3.61. Found: C, 52.70; H, 6.40; N, 3.71.

Methyl 5-Acetamido-4-*O*-acetyl-2,3,5-trideoxy-D-*glycero*-D-*galacto*-2-enopyranosonate (10). A solution of **9** (1.04 g, 2.69 mmol) in 80% acetic acid (20 mL) was heated at 60 °C for 1 h and then evaporated in vacuo. Column chromatography of the residue ($CHCl_3$/MeOH, 93:7) afforded **10** (880 mg, 94%) as an amorphous mass: $[\alpha]_D$ 51.9° (*c* 2, MeOH); ^{1}H NMR (250 MHz, CD_3OD) δ 1.97, 2.06 (s, 3 H, Ac), 3.58 (dd, 1 H, $J_{6,7}$ = 1.2, $J_{7,8}$ = 9.1 Hz, 7-H), 3.66 (m, 1 H, 9a-H), 3.78 (s, 1 H, COOMe), 3.82 (m, 1 H, 9b-H), 3.88 (m, 1 H, 8-H), 4.27 (dd, 1 H, $J_{4,5}$ = 8.6, $J_{5,6}$ 11.3 Hz, 5-H), 4.35 (dd, 1 H, $J_{5,6}$ = 11.3, $J_{6,7}$ = 1.2 Hz, 6-H), 5.65 (dd, 1 H, $J_{3,4}$ = 2.6, $J_{4,5}$ = 8.6 Hz, 4-H), 5.88 (d, 1 H, $J_{3,4}$ = 2.6 Hz, 3-H). Anal. Calcd for $C_{14}H_{21}NO_9$: C, 48.41; H, 6.05; N, 4.03. Found: C, 48.37; H, 6.00; N, 4.05.

Methyl 5-Acetamido-4-*O*-acetyl-9-*O*-benzyl-2,3,5-trideoxy-D-*glycero*-D-*galacto*-non-2-enopyranosonate (7). A mixture of **10** (880 mg, 2.54 mmol) and Bu_2SnO (695 mg, 2.79 mmol) in toluene (38 mL) and methanol (2 mL) was heated at 85 °C for 2 h. The solvents were evaporated, tetrabutylammonium bromide (900 mg, 2.79 mmol), benzyl bromide (0.83 mL, 7 mmol), and toluene (20 mL) were added, and the mixture was heated at 80 °C for 4 h. The solution was evaporated in vacuo, and the residue was purified by column chromatography (toluene/acetone, 7:3) to give **7** (760 mg, 70%) as a white powder which crystallized from diethyl ether: mp 141−143 °C; $[\alpha]_D$ 55.8° (*c* 2, $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.99, 2.08 (s, 3 H, Ac), 3.54 (dd, 1 H, $J_{6,7}$ < 1, $J_{7,8}$ = 9.2 Hz, 7-H), 3.63 (m, 1 H, 9a-H), 3.74 (s, 3 H, COOMe), 3.82 (m, 1 H, 9b-H), 4.13 (m, 1 H, 8-H), 4.16 (dd, 1 H, $J_{5,6}$ = 11.4, $J_{6,7}$ < 1 Hz, 6-H), 4.23 (m, 1 H, 5-H), 4.52, 4.57 (m, 2 H, OCH_2Ph), 5.64 (dd, 1 H, $J_{3,4}$ = 2.6, $J_{4,5}$ = 8.8 Hz, 4-H), 5.82 (d, 1 H, $J_{3,4}$ = 2.6 Hz, 3-H), 6.16 (d, 1 H, $J_{5,NH}$ = 9.1 Hz, NH), 7.50 (m, 5 H, Ph). Anal. Calcd for $C_{21}H_{26}NO_9$: C, 57.79; H, 5.96; N, 3.21. Found: C, 57.80; H, 5.92; N, 3.20.

Methyl 5-Acetamido-4-*O*-acetyl-9-*O*-benzyl-2,3,5-trideoxy-8-*O*-{methyl [5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid]onate}-D-*glycero*-D-*galacto*-non-2-enopyranosonate (14α). Method A. To a stirred mixture of **7** (200 mg, 0.46 mmol), halogenose **3e** (372 mg, 0.55 mmol), and molecular sieves (500 mg) in dry 1,2-dichloroethane (5 mL) was added silver

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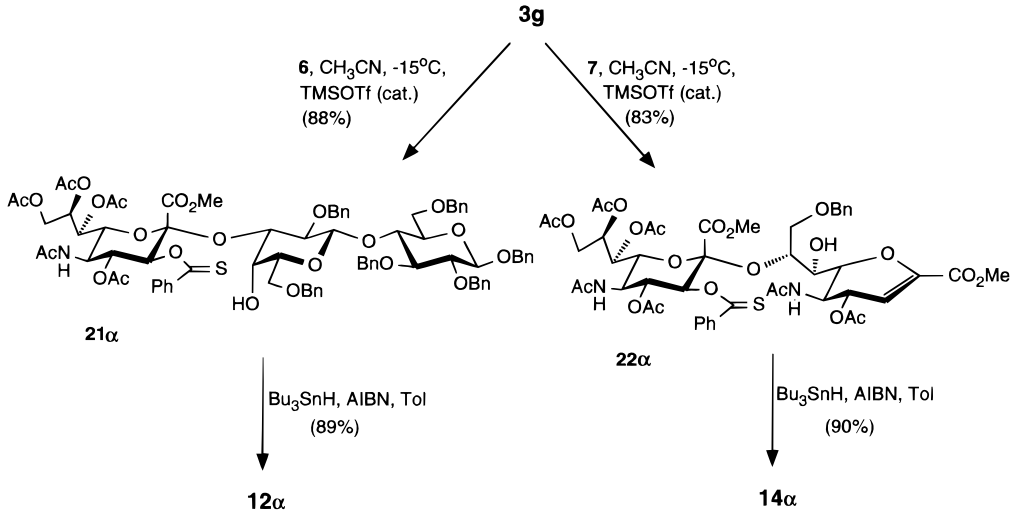
Scheme 6



triflate (141 mg, 0.55 mmol) at −10 °C under an argon atmosphere. The mixture was stirred for 1 h and filtered, and the solid was washed with chloroform. The combined filtrates and washings were evaporated in vacuo to afford **13**α, which was immediately used in the next step. To a solution of **13**α in toluene (5 mL) were added tributylstannane (0.37 mL, 1.38 mmol) and AIBN (catalytic amount) under an argon atmosphere. The mixture was heated at 110 °C for 3 h and then evaporated to dryness. Column chromatography of the residue (toluene/acetone, 7:3) afforded **14**α (273 mg, 65%) as a white foam.

Method B. A solution of acceptor **7** (200 mg, 0.46 mmol) and donor **3g** (420 mg, 0.55 mmol) in dry acetonitrile (5 mL) was cooled to −15 °C. To this solution was added trimethylsilyl triflate (20 μL, 0.11 mmol). After 3 h the reaction was allowed to reach room temperature, neutralized with triethylamine, and then concentrated. The residue was washed with 1 N HCl solution (2×) and water (3×), dried ($MgSO_4$), and concentrated to afford **22**α, which was immediately used in the next step. To a solution of the residue in toluene (5 mL) were added tributylstannane (0.37 mL, 1.38 mmol) and AIBN (catalytic amount) under an argon atmosphere. The mixture was heated at 110 °C for 3 h and then evaporated to dryness. Column chromatography of the residue (toluene/acetone, 7:3) afforded **14**α (315 mg, 74%) as a white foam: $[\alpha]_D$ 15° (*c* 1, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.80−2.10 (6 s. 18 H, 6 Ac), 2.44 (dd, 1 H, $J_{3ax,3eq}$ = 12.3, $J_{3ax,4}$ = 11.4 Hz, 3′$_{ax}$-H), 2.60 (dd, $J_{3eq,3ax}$ = 12.3, $J_{3eq,4}$ = 4.6 Hz, 3′$_{eq}$-H), 3.56 (m, 1 H, 9a-H), 3.77 (dd, 1 H, $J_{5,6}$ = 11.0, $J_{6,7}$ = 2.3 Hz, 6′-H), 3.84 (m, 1 H, 9b-H), 3.91 (m, 2 H, 5′-H, 9′a-H), 4.31 (m, 2 H, 5-H, 6-H), 4.52 (m, 1 H, 9′b-H), 4.57 (m, 1 H, 8-H), 4.89 (m, 1 H, 4′-H), 5.19 (m, 1 H, 8′-H), 5.30 (dd, 1 H, $J_{6,7}$ = 2.3, $J_{7,8}$ = 4.6 Hz, 7′-H), 5.34 (d, 1 H, $J_{NH,5}$ = 9.8 Hz, NH), 5.76 (dd, 1 H, $J_{3,4}$ = 2.5, $J_{4,5}$ = 9.2 Hz, 4-H), 5.92 (d, 1 H, $J_{3,4}$ = 2.5 Hz, 3-H), 6.91 (d, 1 H, $J_{5,NH}$ = 8.3 Hz, NH′); ^{13}C NMR (150 MHz, $CDCl_3$) δ 20.41, 20.73, 20.78, 20.98, 21.02, 22.96, 22.99 (7 *C*H_3CO), 34.54, (C-3′), 47.42 (C-5), 48.70 (C-5′), 63.52 (C-9′), 67.92 (C-7′), 68.25 (C-7), 68.65 (C-4), 69.94 (C-4′), 70.48 (C-8′), 70.80 (C-9), 72.96 (C-8), 73.53 (C-6′), 76.80 (C-6), 100.61 (C-2′), 107.26 (C-3), 145.85 (C-2), 161.97 (*C*OOMe), 167.83 (*C*OOMe′). Anal. Calcd for $C_{41}H_{52}N_2O_{21}$: C, 54.17; H, 5.72; N, 3.08. Found: C, 54.20; H, 5.71; N, 3.07.

Methyl 5-Acetamido-4-*O*-acetyl-9-*O*-benzyl-2,3,5-trideoxy-8-*O*-{methyl [5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-*β*-D-*galacto*-2-nonulopyranosid]onate}-D-*glycero*-non-2-enopyranosonate (14*β*). A solution of acceptor **7** (200 mg, 0.46 mmol) and donor **3f** (370 mg, 0.55 mmol) in dry dichloromethane (5 mL) was cooled to −20 °C. To this solution was added trimethylsilyl triflate (20 μL, 0.11 mmol). After 3 h the reaction was allowed to reach room temperature, neutralized with triethylamine, and then concentrated. The residue was washed with 1 N HCl solution (2×), and water (3×), dried ($MgSO_4$), and concentrated to give **17***β*, which was immediately used in the next step. To a solution of **17***β* in toluene (5 mL) were added tributylstannane (0.37 mL, 1.38 mmol) and AIBN (catalytic amount) under an argon atmosphere. The mixture was heated at 110 °C for 3 h and then evaporated to dryness. Column chromatography of the residue (toluene/acetone, 7:3) afforded **12***β* (231 mg, 55%) as a white foam: $[\alpha]_D$ 18° (*c* 1, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.70 (dd, $J_{3ax,3eq}$ = 13.1, $J_{3ax,4}$ = 11.8 Hz, 3′$_{ax}$-H), 1.82−2.15 (7 s, 21 H, 7 Ac), 2.35 (dd, 1 H, $J_{3ax,3eq}$ = 13.1, $J_{3eq,4}$ = 4.5 Hz, 3′$_{eq}$-H), 3.59 (m, 1 H, 9a-H), 3.73 (m, 1 H, 8-H), 3.76 (m, 1 H, 9b-H), 3.94 (dd, $J_{6,7}$ < 1, $J_{7,8}$ = 8.7 Hz, 7-H), 3.97 (dd, $J_{4,5}$ = 11, $J_{5,6}$ = 10.5 Hz, 5′-H), 4.13 (m, 1 H, 9′a-H), 4.23 (dd, $J_{6,7}$ < 1, $J_{5,6}$ = 11.0 Hz, 6-H), 4.27 (dd, 1 H, $J_{5,6}$ = 11.0, $J_{4,5}$ = 8.7 Hz, 5-H), 4.44 (dd, 1 H, $J_{5,6}$ = 10.5, $J_{6,7}$ = 2 Hz, 6′-H), 4.47 (m, 1 H, 9′b-H), 4.94 (m, 1 H, 4′-H), 5.30 (m, 1 H, 8′-H), 5.44 (dd, 1 H, $J_{6,7}$ = 2.0, $J_{7,8}$ = 7.2 Hz, 7′-H), 5.62 (dd, 1 H, $J_{3,4}$ = 2.5, $J_{4,5}$ = 8.7 Hz, 4-H), 5.89 (d, 1 H, $J_{3,4}$ = 2.5 Hz, 3-H), 6.18 (d, 1 H, $J_{5,NH}$ = 7.5 Hz, NH), 6.34 (d, 1 H, $J_{5,NH}$ = 10.2 Hz, NH); ^{13}C NMR (150 MHz, $CDCl_3$) δ 20.71, 20.75, 20.79, 20.92, 20.98, 23.05 (7 *C*H_3-CO), 38.83 (C-3′), 48.75 (C-5), 48.87 (C-5′), 62.05 (C-9′), 66.17 (C-7′), 68.35 (C-7), 68.43 (C-4), 69.95 (C-4′), 71.83 (C-8′), 72.51 (C-9), 72.73 (C-8), 72.80 (C-6′), 77.54 (C-6), 96.87 (C-2′), 108.16 (C-3), 145.36 (C-2), 163.07 (*C*OOMe), 168.64 (COOMe′). Anal. Calcd for $C_{41}H_{52}N_2O_{21}$: C, 54.17; H, 5.72; N, 3.08. Found: C, 54.15; H, 5.69; N, 3.08.

Methyl 5-Acetamido-5-deoxy-4,7,8,9-tetra-*O*-acetyl-*β*-D-*erythro*-L-*gluco*-2-nonulopyranosonate (18). To a solution of **1** (10 g, 0.02 mmol) in a mixture of acetone−water−*tert*-butyl alcohol (3:1:0.1, 50 mL) were added osmium tetroxide (100 mg, 0.39 mmol) and *N*-methylmorpholine *N*-oxide (3.51 g, 0.03 mol). After 10 h, saturated sodium bisulfite solution was added, and the mixture was stirred for 30 min. The solid was filtered off, to the resultant clear solution was added water (50 mL) was added, and extraction with ethyl acetate (5 × 20 mL) was performed. The organic extracts were dried ($MgSO_4$), filtered, and concentrated to dryness. Column chromatography of the residue (toluene/acetone, 2:1) afforded **18** (10.2 g, 95%) as a white foam: $[\alpha]_D$ −62° (*c* 0.9, $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.80−2.15 (5 s, 15 H, 5 Ac), 3.05 (br s, 1 H, 3-OH), 3.81 (s, 3 H, COOMe), 3.92 (m, 1 H, 9a-H), 4.04 (d, 1 H, $J_{3,4}$ = 9.5 Hz, 3-H), 4.10−4.30 (m, 2 H, 5-H, 6-H), 4.45 (m, 1 H, 9b-H), 5.01 (dd, 1 H, $J_{3,4}$ = 9.5, $J_{4,5}$ = 9.2 Hz, 4-H), 5.18 (m, 1 H, 8-H), 5.30 (dd, 1 H, $J_{6,7}$ = 1.4, $J_{7,8}$ = 8.6 Hz, 7-H), 5.45 (br s, 1 H, 2-OH), 6.45 (d, 1 H, $J_{5,NH}$ = 9.5 Hz, NH). Anal. Calcd for $C_{20}H_{28}NO_{14}$: C, 47.31; H, 5.52; N, 2.76. Found: C, 47.20; H, 0.5.50; N, 2.73.

Methyl 5-Acetamido-4,7,8,9-tetra-*O*-acetyl-5-deoxy-3-*O*-thiobenzoyl-*β*-D-*erythro*-L-*gluco*-2-nonulopyranosonate (20). A solution of *N*,*N*-dimethyl-α-chlorobenzimidium chloride (made from 1 g of *N*,*N*-dimethylbenzamide and 1 g of phosgene)[21] in dry dichloromethane (5 mL) was added dropwise to a solution of diol **18** (1 g, 1.96 mmol) in dichloromethane, and the mixture was stirred for 0.5 h at room temperature. Pyridine (1 mL) was then added, and the solution was treated for 15 min with hydrogen sulfide. The mixture was evaporated in vacuo, and the residue was purified by column chromatography

(toluene/acetone, 2:1) to afford **20** (1.16 g, 92%) as a pale yellow syrup: $[\alpha]_D$ −57° (*c* 1, $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.80−2.15 (5 s, 15 H, 5 Ac), 3.80 (s, 3 H, COOMe), 4.03 (m, 1 H, 9a-H), 4.38 (dd, 1 H, $J_{5,6}$ = 8.8, $J_{6,7}$ = 1.4 Hz, 6-H), 4.44 (m, 2 H, 9b-H, 5-H), 5.23 (m, 2 H, 8-H, OH), 5.39 (dd, 1 H, $J_{6,7}$ = 1.4, $J_{7,8}$ = 9.0 Hz, 7-H), 5.44 (dd, 1 H, $J_{3,4}$ = 9.4, $J_{4,5}$ = 9.1 Hz, 4-H), 5.59 (d, 1 H, $J_{3,4}$ = 9.4 Hz, 3-H), 6.42 (d, 1 H, $J_{NH,5}$ = 9.1 Hz, NH), 7.20−7.85 (m, 5 H, Ph). Anal. Calcd for $C_{27}H_{32}NO_{14}S$: C, 51.34; H, 5.11; N, 2.23. Found: C, 50.53, H, 4.99; N, 2.18.

Methyl 5-Acetamido-4,7,8,9-tetra-*O*-acetyl-2-*O*-(diethyl phosphite)-3-*O*-thiobenzoyl-*β*-D-*erythro*-L-*gluco*-2-nonulopyranosonate (3g). To a solution of **20** (500 mg, 0.78 mmol) in dry acetonitrile (8 mL) were added Hünig's base (0.23 mL, 1.33 mmol) and diethyl chlorophosphite (0.17 mL, 1.17 mmol) at room temperature. After 1 h, the solution was evaporated in vacuo, and the residue was washed with saturated aqueous sodium hydrogenocarbonate solution and water, dried ($MgSO_4$), and concentrated. The residue was chromatographed on silica gel (toluene/acetone, 2.5:1) to afford **3g** (546 mg, 92%) as a white foam: $[\alpha]_D$ −42° (*c* 1, $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.01−1.09 (m, 6 H, 2 OCH_2CH_3), 1.71−2.10 (5 s, 15 H, 5 Ac), 3.61 (s, 3 H, COOMe), 3.70−4.09 (m, 4 H, 2 OCH_2CH_3), 4.10 (m, 1 H, 9a-H), 4.21 (m, 2 H, 5-H, 6-H), 4.40 (m 1 H, 9b-H), 5.25 (dd, 1 H, $J_{6,7}$ = 1.7, $J_{7,8}$ = 8.6 Hz, 7-H), 5.35 (m, 2 H, 3-H, 4-H), 5.55 (d, 1 H, $J_{NH,5}$ = 9.0 Hz, NH), 7.20−7.90 (m, 5 H, Ph). Anal. Calcd for $C_{31}H_{41}NO_{16}PS$: C, 49.85; H, 5.49; N, 1.87. Found: C, 49.92; H, 5.50; N, 1.89.

Benzyl 2,3,6-Tri-*O*-benzyl-4-*O*-[2,6-di-*O*-benzyl-3-*O*-(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-5-deoxy-3-*O*-thiobenzoyl-D-*erythro*-α-L-*gluco*-2-nonulopyranosylate)-*β*-D-galactopyranosyl]-*β*-D-glucopyranoside (21α). A solution of **6** (406 mg, 0.46 mmol) and donor **3g** (420 mg, 0.55 mmol) in dry acetonitrile (5 mL) was cooled to −15 °C. To this solution was added trimethylsilyl triflate (20 μL, 0.11 mmol). After 3 h the reaction was allowed to reach room temperature, neutralized with triethylamine and then concentrated. The residue was purified by column chromatography (toluene/acetone, 3:1) to afford **21**α (609 mg, 88%) as a white foam: $[\alpha]_D$ 1.5° (*c* 1, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.79−2.09 (5 s, 15 H, 5 OAc), 3.13 (m, 1 H, 5′-H), 3.25 (m, 1 H, 5a-H), 3.36 (dd, 1 H, $J_{2,3}$ = 9.0, $J_{3,4}$ < 1 Hz, 3′-H), 3.42 (m, 2 H, 2a-H, 6′a-H), 3.47 (m, 2 H, 2′-H, 3-H), 3.63 (m, 2 H, 6a-H, 6′b-H), 3.76 (dd, 1 H, $J_{5,6}$ = 8.9, $J_{6,7}$ = 1.1 Hz, 6″-H), 3.68 (m, 1 H, 6b-H), 3.83 (s, 3 H, COOMe), 3.88 (dd, 2 H, $J_{3,4}$= $J_{4,5}$ = 9.3 Hz, 4-H), 3.96 (dd, 1 H, $J_{3,4}$ = $J_{4,5}$ < 1 Hz, 4′-H), 4.02 (m, 1 H, 9″a-H), 4.21 (m, 1 H, 5″-H), 4.26 (d, 1 H, $J_{1,2}$ = 9.2 Hz, 1′-H), 4.33 (m, 1 H, 9″b-H), 4.41 (d 1 H, $J_{1,2}$ = 9.3 Hz, 1-H), 5.08 (dd, 1 H, $J_{3,4}$, $J_{4,5}$ = 9.4 Hz, 4″-H), 5.14 (m, 1 H, 8″-H), 5.20 (d, 1 H, $J_{3,4}$ = 9.5 Hz, 3″-H), 5.24 (m, 2 H, 7″-H, NH), 7.15−7.33 (m, 35 H, 7 Ph). Anal. Calcd for $C_{81,}H_{75}NO_{25}S$: C, 65.09; H, 5.02; N, 0.93. Found: C, 65.00; H, 5.05, N, 1.01.

Acknowledgment. This work was supported by the Deutsche Forschungsgemeinschaft and the Fonds der Chemischen Industrie. J.C.C.-P. is grateful for a DAAD stipend. We are grateful to Dr. Armin Geyer for his help in the structural assignments by NMR experiments.

Supporting Information Available: Synthesis and characterization details for **3e**, **3f**, **7**, **11**α, **12**α, **15**α, **16**α, **18**, and **19** (5 pages, print/PDF). See any current masthead page for ordering information and Web access instructions.

JA9740327