Solution Phase Synthesis of Amide-Linked *N*-Acetyl Neuraminic Acid, α-Amino Acid, and Sugar Amino Acid Conjugates[1]

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Peracetylated *N*-acetylneuraminic acid (NeuAc) was efficiently coupled to esters of glycine, alanine, and serine using BOP and HOBT in the presence of DIEA. Deprotection of the esters readied the NeuAc-α-amino acid conjugates for further elaboration. Coupling of the NeuAc-gly adduct with *β*-*O*-methoxy neuraminic acid methyl ester afforded a selectively protected glycine linked sialic acid dimer. 2-Amino-3,4-di-*O*-benzyl-(1→6)-anhydroglucose and alanine benzyl ester were also efficiently coupled to the bis adduct giving novel trimeric analogs. Elimination of the anomeric acetate from the NeuAc-gly dimer followed by global deprotection provided a novel saccharopeptide with modest *clostridial* sialidase inhibitory activity.

Introduction

Combinatorial chemistry has become an important tool in the pharmaceutical industry for developing new lead compounds possessing desired biological activity. One way of generating diversity in small molecule combinatorial libraries is to use a wide variety of naturally occurring building blocks. This approach has been used successfully to create large libraries of peptides and oligonucleotides.[2] Carbohydrates are biologically important molecules and provide an untapped source for generating combinatorial libraries. Due to the density of functional groups in a typical monosaccharide, and the choice of stereochemical linkage at the anomeric carbon, one can in principle synthesize a large number of disaccharides from just two monomer units. Assembly of oligosaccharides through glycosidic linkages suffers from two major disadvantages. The stereochemical outcome of glycosidation reactions is not always predictable, and the oligosaccharides thus prepared are susceptible to biodegradation through the action of glycosidases. While progress has been made in solid phase synthesis of oligosaccharides,[3] it is not yet a routine procedure. A potential solution to the aforementioned problems involves suitably modifying the monosaccharides so that they can be readily coupled to each other and to other classes of molecules. A number of research groups, including our own, have successfully incorporated both an amine and carboxylic acid functionality into monosaccharides to provide so-called sugar amino acids (SAA).[4] These modified sugars can then be coupled by standard, solution phase peptide coupling reactions to yield amide-linked oligosaccharides.[5,6]

In addition to assembling SAA's to afford amide-linked oligosaccharides, one can envision coupling them to other naturally occurring building blocks to generate diverse sets of chemical libraries. For example, adducts of sugar amino acids with naturally occurring α-amino acids would yield a class of molecules which we refer to as saccharopeptides.[7] These different classes of molecules derived from SAA have considerable potential apart from their novelty. Besides providing new small molecule inhibitors (*vide infra*), the well defined conformations of carbohydrates and the derived SAA lend themselves to the rational design of new oligosaccharide mimetics[5i,j] and peptidomimetics[5g] and to the discovery of novel materials with ordered secondary structures.[8] Furthermore, the presence of multiple hydroxy groups on SAA facilitates their solubility in aqueous solvents, and as peptidomimetics they may be resistant to proteases because of their modified backbone.

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(1) Presented at the 213th National ACS Meeting, San Francisco, CA, 1997 ORGN 579.

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(3) Rademann, J.; Schmidt, R. R. *J. Org. Chem.* **1997**, *62*, 3650 and references cited therein.

(4) To our knowledge the first example of a synthetic SAA was reported by Paulsen in 1955. Heyns, K.; Paulsen, H. *Chem. Ber.* **1955**, *88*, 188.

(5) The first synthesis of amide-linked sugars from SAA was reported by Fuchs and Lehmann. (a) Fuchs, E.-F.; Lehmann, J. *Chem. Ber.* **1975,** *108*, 2254. (b) Fuchs, E.-F.; Lehmann, J. *Carbohydr. Res.* **1976**, *49*, 267. (c) Yoshimura, J.; Ando, H.; Sato, T.; Tsuchida, S.; Hashimoto, H. *Bull. Chem. Soc. Jpn.* **1976**, *49*, 2511. (d) Apostopoulos, C. D.; Couladouros, E. A.; Georgiadis, M. P. *Liebigs Ann. Chem.* **1994**, 781. (e) Kessler, H.; Graf von Roedern, E. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 687. (f) Frey, O.; Hoffmann, M.; Kessler, H. *Angew. Chem. Int., Ed. Engl.* **1995**, *34*, 2026. (g) Graf von Roedern, E.; Lohof, E.; Hessler, G.; Hoffmann, M.; Kessler, H. *J. Am. Chem. Soc.* **1996**, *118*, 10156. (h) Poitout, L.; Le Merrer, Y.; Depezay, J.-C. *Tetrahedron. Lett.* **1995**, *36*, 6887. (i) Muller, C.; Kitas, E.; Wessel, H. P. *J. Chem. Soc., Chem. Commun.* **1995**, 2425. (j) Wessel, H. P.; Mitchell, C. M.; Lobato, C. M.; Schmid, G. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 2712. (k) Suhara, Y.; Hildreth, J. E. K.; Ichikawa, Y. *Tetrahedron Lett.* **1996**, *37*, 1575. (l) Suhara, Y.; Ichikawa, M.; Hildreth, J. E. K.; Ichikawa, Y. *Tetrahedron. Lett.* **1996**, *37*, 2549. (m) McDevitt, J. P.; Lansbury, P. T., Jr. *J. Am. Chem. Soc.* **1996**, *118*, 3818. (n) Gervay, J.; Flaherty, T. M.; Nguyen, C. *Tetrahedron Lett.* **1997**, *38*, 1493. (o) Goodnow, R. A., Jr.; Richou, A.-R.; Tam, S. *Tetrahedron Lett.* **1997**, *38*, 3195. (p) Goodnow, R. A., Jr.; Tam, S.; Pruess, D. L.; McComas, W. W. *Tetrahedron Lett.* **1997**, *38*, 3199. (q) Sabesan, S. *Tetrahedron. Lett.* **1997**, *38*, 3127.

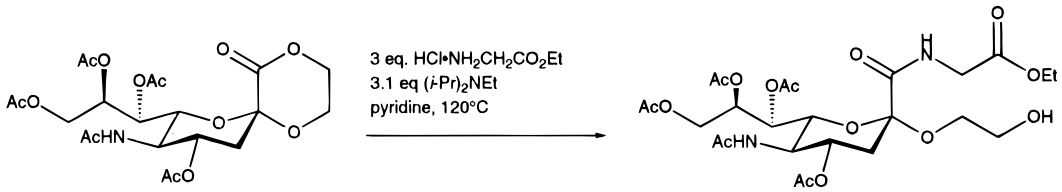
(6) (a) Kanie, O.; Barresi, F.; Ding, Y.; Labbe, J.; Otter, A.; Forsberg, L. S.; Ernst, B.; Hindsgaul, O. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 2720. (b) Liang, R.; Yan, L.; Loebach, J.; Ge, M.; Yozumi, Y.; Sekanina, K.; Horan, N.; Gildersleeve, J.; Thompson, C.; Smith, A.; Biswas, K.; Still, W. C.; Kahne, D. *Science* **1996**, *274*, 1520.

(7) A plethora of terms have been proposed in the literature for compounds derived from SAA. These include carbopeptoids, saccharide-peptide hybrids, glycosamino acids, peptidosaccharides, saccharopeptides, and amide-linked carbohydrates, to name a few. In some cases, the SAA are linked to each other to give compounds that are completely carbohydrate-based. In other cases, they are linked to naturally occurring amino acids. In order to readily distinguish between the two possibilities, we refer to the former class as amide-linked sugars and the latter class as saccharopeptides. The term saccharopeptide itself was, to our knowledge, first proposed by Fugedi to describe compounds that we now refer to as amide-linked sugars. See Fugedi, P.; Peto, Cs; Wlasichuk, K. 8th European Carbohydrate Symposium, July 2−7, 1995, Absract A74−75.

(8) Appella D. H.; Chrstianson, L. A.; Karle, I. L.; Powell, D. R.; Gellman, S. H. *J. Am. Chem. Soc.* **1996**, *118*, 13071.

S0022-3263(97)01124-9 CCC: $14.00

Scheme 1



2 **3**

Results and Discussion

Our interest in NeuAc derived SAA began with our work directed toward the design and synthesis of neuraminidase inhibitors.[9] Our original target was the preparation of *N*-acetyl neuraminic acid (NeuAc) analogs that had an extended spacer unit between C-1 and C-2. We achieved this by the ring-opening of a spirocyclic sialyllactone (**2**) with glycine ethyl ester to obtain the glycosylated glycinyl-NeuAc saccharopeptide **3** (Scheme 1).

The yields in these reactions were moderate, and we realized that this method could not be extended to more sterically hindered amino acids. Furthermore, the spirolactone **2** was prepared through a sequence involving a glycosylation. A general problem in glycosylation reactions of NeuAc derivatives is the formation of the 2,3-didehydro compounds via elimination. This lowers the yield of the reaction and can cause difficulties during purification. Therefore, we sought a more general route for the construction of amide-linked sugars. In this report, we outline a general method for the synthesis of saccharopeptides derived from NeuAc. We chose to work with NeuAc because it is an important component of cell surface glycoconjugates involved in cellular recognition processes, and synthesis of small molecule analogs of NeuAc is an important area of research.[10] We also anticipated that the extended side chain of NeuAc would increase the water solubility of the conjugates, potentially providing saccharopeptides with greater bioavailability.

The first challenge was to establish efficient coupling conditions for amidation of NeuAc. In our initial investigations, NeuAc was reacted with acetic anhydride in pyridine to afford the peracetylated β-acetate **4**[11] (>95:5 β:α) which was used for subsequent reactions. Attempted coupling of **4** using dicyclohexylcarbodiimide (DCC) in pyridine did not lead to the desired products, presumably due to the sterically demanding neopentyl carboxylic acid. However, we were pleased to find that (benzotriazol-1-yloxy)-tris(dimethylamino)phosphonium hexafluorophosphate (BOP reagent) worked quite well. The first coupling was attempted with a slight excess of glycine ethyl ester using the BOP reagent in DMF. After aqueous workup and extraction into ethyl acetate, the ^{1}H NMR indicated that the desired coupling product had formed, but the yield was moderate (35%). We concluded that this was most likely due to poor extraction of the product into the organic layer. After experimenting with a number of different conditions, we found that coupling with 1.1 equiv of BOP, 1.1 equiv of 1-hydroxybenzotriazole hydrate (HOBT), 4 equiv of *N*,*N*-diisopropylethylamine (DIEA), and a slight excess of glycine ethyl ester hydrochloride (1.3−1.5 equiv) in 4:1 CH_2Cl_2/DMF was optimum. After flash column, a 60% yield of the coupled product **5** was obtained.[12] The crude yield of the reaction after extraction was >95% and very clean. It was subjected to flash column chromatography only for characterization purposes. Coupling of **4** with serine benzyl ester hydrochloride gave the desired product **6** in 44% yield after flash column. Similarly, coupling with alanine benzyl ester hydrochloride gave the desired product **7** in 60% yield (Scheme 2). No racemization was observed in the latter cases as evidenced by ^{1}H and ^{13}C NMR. Once again, the crude yields were >95%, and the spectra were very clean. The only other product observed in these cases was the adduct derived from the coupling of the minor α anomer of **4**.

We next turned our attention to elaborating these disaccharopeptides. We chose benzyl esters of the α-amino acids because they could be selectively cleaved in the presence of the acetate protecting groups on the NeuAc moiety. The resulting free acids could then be coupled again to yield trimeric saccharopeptide analogs. In the event, **4** was reacted with glycine benzyl ester hydrochloride to give the desired product **8** in 65% yield after flash column chromatography. The benzyl ester was subsequently removed by hydrogenation over Pd/C to give compound **9** in quantitative yield (Scheme 3). As mentioned before, the product from the initial coupling was sufficiently pure after an extractive workup, and by directly subjecting crude **8** to hydrogenation, **9** was obtained in 80−90% overall yield from peracetylated NeuAc (**4**).

The first compound we attempted to couple to **9** was the SAA β-*O*-methoxy neuraminic acid methyl ester (**10**) which would provide a differentially protected sialic acid dimer linked by a glycine spacer. NeuAc is the most abundant form of the naturally occurring sialic acids, and it must be deacetylated to be used as a SAA. Recently, we outlined a convenient procedure for *N*-deacylation of NeuAc that is suitable for the preparation of a variety of sialic acid derivatives.[5n] Compound **10** was thus prepared and subjected to coupling with **9** using the BOP reagent as described above. The desired product **11** was obtained in low yield, with the major product being an unidentified polymer (Scheme 4). To determine which component was unstable under the reaction conditions, **9** was coupled to alanine benzyl ester hydrochloride, and the trimeric species **12** was obtained in 54% yield,

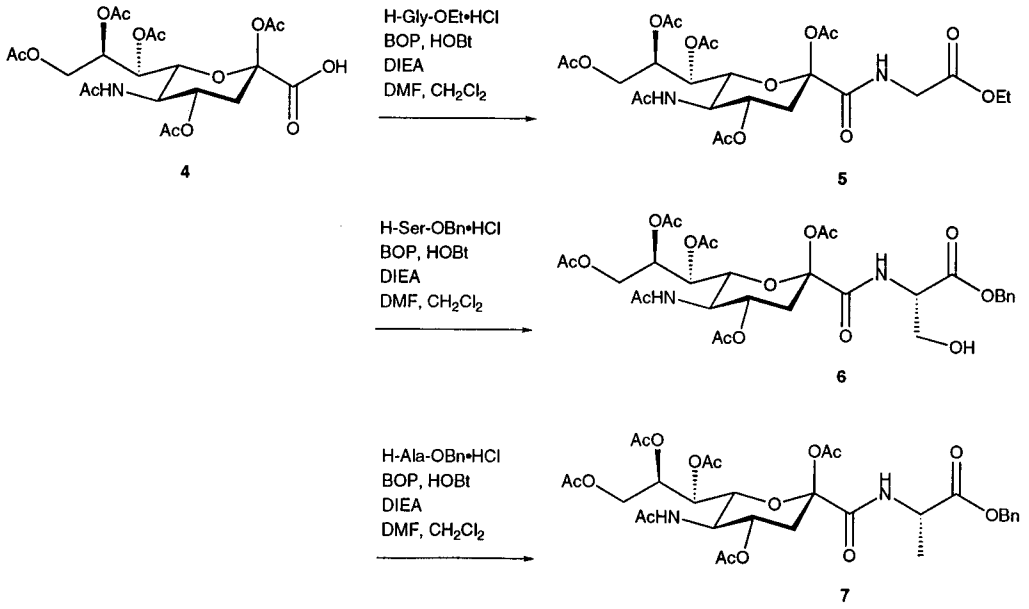
(9) Gervay, J.; Ramamoorthy, P. S.; Mamuya, N. M. *Tetrahedron* **1997**, *53*, 11039.

(10) For lead references see (a) Kim, C. U.; Lew, W.; Williams, M. A.; Liu, H.; Zhang, L.; Swaminathan, S.; Bischofberger, N.; Chen, M. S.; Mendel, D. B.; Tai, C. Y.; Laver, W. G.; Stevens, R. C. *J. Am. Chem Soc.* **1997**, *119*, 681. (b) Sabesan, S.; Neira, S.; Davidson, F.; Duus, J. O.; Bock, K. *J. Am. Chem. Soc.* **1995**, *116*, 1616. (c) von Itzstein, M.; Wu, W.; Kok, G. B.; Pegg, M. S.; Dyason, J. C.; Jin, B.; Phan, T. V.; Smythe, M. L.; White, H. F.; Oliver, S. W.; Colman, P. M.; Varghese, J. N.; Ryn, D. M.; Woods, J. M.; Bethell, R. C.; Hotham, V. J.; Cameron, J. M. and Penn, C. R. *Nature (London)* **1993**, *363*, 418.

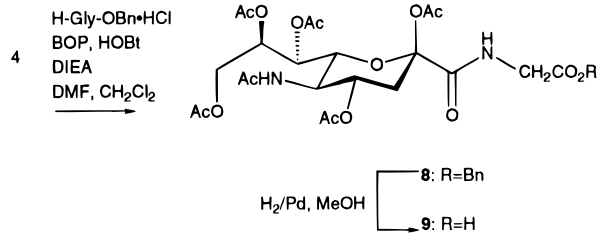
(11) Meyers, R. W.; Lee, R. T.; Lee, Y. C.; Thomas, G. H.; Reynolds, L. W.; Uchida, Y. *Anal. Biochem.* **1980**, *101*, 166

(12) Leaving out HOBT does not affect the yield of the reaction. It was used to suppress racemization.

Scheme 2



Scheme 3



suggesting that **10** may be undergoing polymerization (Scheme 5).[13]

In order to demonstrate the generality of the coupling method, coupling of **9** to glucosamine hydrochloride (**13**) was attempted. Upon addition of DIEA to the reaction mixture, the reaction turned black and a polymeric material was obtained. The reaction was also attempted using ethyl acetate as solvent with equally disappointing results. Both 2- and 3-amino sugars are known to undergo complex rearrangements under basic conditions, and this was presumably the case under our reaction conditions (Scheme 6).[14]

To circumvent this problem, the 1,6-anhydro glucosamine derivative **14** was prepared in six steps from tri-*O*-acetyl-D-glucal according to the literature procedure.[15] Treatment of **9** with **14** under the usual conditions resulted in 58% yield of the desired product **15** after purification. A small amount of the *N*-acetylated glucosamine derivative was also isolated from the reaction mixture. The acetate group on the nitrogen is presumably derived from the NeuAc moiety by attack of the amine. Deprotection of anomeric acetates by amines is precedented in carbohydrate literature.[16] However, this is not a general problem and can be eliminated by the use of nonelectrophilic protecting groups such as benzyl ethers and acetals. This coupling example is particularly noteworthy because **14** has a sterically demanding axial amine functionality. Another advantage of using the anhydro sugar is that one can selectively manipulate the (1→6)-linkage to provide functionality suitable for further amide coupling and/or glycosylations.

In order to explore the potential of these saccharopeptides as neuraminidase inhibitors, compound **5** was subjected to elimination with trimethylsilyl triflate (TMSOTf) to give a 2,3-dehydro derivative in 40% yield (Scheme 7). Global deprotection of the compound with sodium methoxide in methanol, followed by treatement with sodium hydroxide in water, gave the fully deprotected saccharopeptide **16**. This compound proved to be a moderate inhibitor of *Clostridial* sialidase.[17]

Conclusions

We have demonstrated that NeuAc can be efficiently conjugated to naturally occurring α-amino acids and subsequently to sugar amino acids providing structurally diverse compounds with potential biological activity. Typical peptide bond forming protocols are employed making this method amenable to solution phase combinatorial techniques. An apparent problem from this study is the difficulty in using unprotected amino sugars in such coupling reactions. While trimeric materials were efficiently prepared in solution, solid phase techniques may be more suitable for larger oligomers. We have recently demonstrated the power of solid phase synthesis in the preparation of octameric (1→5) amide-linked sialooligomers.[18] The use of both solution and solid phase methodologies in the synthesis of novel oligosaccharide mimetics and peptidomimetics are the focus of current investigations in our laboratories.

Experimental Section

General Methods. All reactions were performed in oven-dried round bottom flasks fitted with rubber septa under a positive pressure of argon, unless otherwise noted. Air- and

(13) We are currently studying the problems associated with coupling reactions involving **10**.

(14) Collins, P.; Ferrier, R. *Monosaccharides: Their Chemistry and Their Roles in Natural Products*; Wiley: Chichester, 1995; p 233.

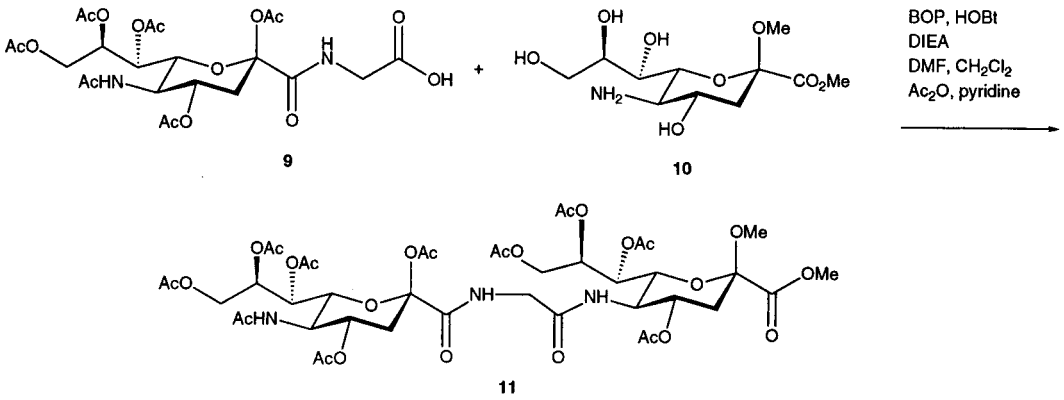
(15) (a) Tailler, D.; Jacquinet, J.-C.; Noirot, A.-M.; Beau, J.-M. *J. Chem. Soc., Perkin Trans. 1* **1992**, 3163. (b) Leteux, C.; Veyrieres, A.; Robert, F. *Carbohydr. Res.* **1993**, *242*, 119.

(16) Avalos, M.; Babiano, R.; Cintas, P.; Jimenez, J. L.; Palacios, J. C.; Valencia, C. *Tetrahedron Lett.* **1993**, *34*, 1359 and references therein.

(17) Full experimental details of this work and the biological testing of this and other compounds will be reported in a forthcoming publication from our laboratory.

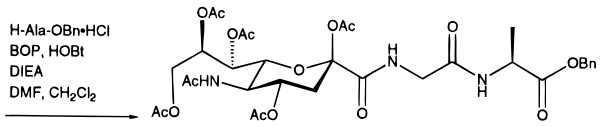
(18) Szabo L.; Smith, B. L.; McReynolds, K. D.; Parrill, A. L.; Morris, E. R.; Gervay, J. *J. Org. Chem.*, submitted for publication.

Scheme 4



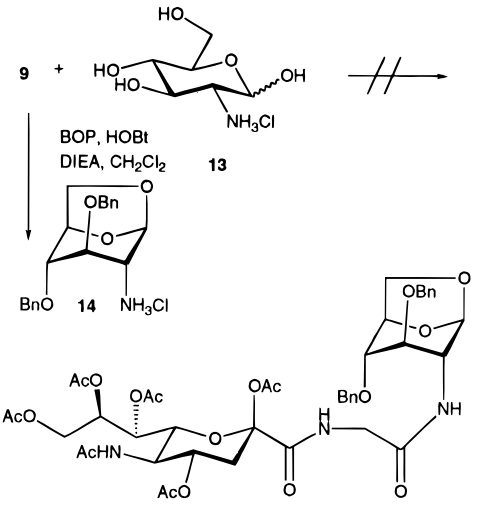
9 10 11

Scheme 5



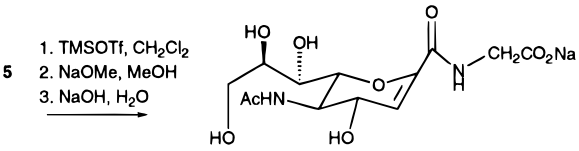
12

Scheme 6



13 14 15

Scheme 7



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moisture-sensitive liquids and solutions were transferred via syringe or stainless steel canula. Organic solutions were concentrated by rotary evaporation at ~10−15 Torr. Flash column chromatography was performed according to the method of Still et al.[19] employing 230−400 mesh silica gel. Analytical thin-layer chromatography was performed using glass plates precoated with 0.25 mm silica gel impregnated with a flourescent indicator (254 nm). Commercial reagents and solvents were used as received with the following exceptions. Dichloromethane and DMF were distilled from CaH_2.

General Procedure for Coupling. To a stirred, 0.2 M solution of the carboxylic acid (1.0 equiv) in 4:1 CH_2Cl_2/DMF was added BOP reagent (1.1 equiv), HOBt (1.1 equiv), diisopropylethylamine (4.0 equiv), and the amine hydrochloride salt (1.3−1.5 equiv). The reaction was stirred at rt for 12−15 h and concentrated on the rotovap. The crude material was dissolved in EtOAc and washed with saturated aqueous $NaHCO_3$, H_2O, and brine. The organic layer was dried with $MgSO_4$ and concentrated. The product was purified by flash chromatography using hexanes/ethyl acetate or hexanes/acetone.

Peracetylated NeuAc-Gly-OEt (5): 60% yield. ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.28 (t, J = 7.2, 3H, OCH_2CH_3), 1.90 (s, 3H, NH*Ac*), 1.91 (m, 1H, H_{3ax}), 2.02 (s, 3H, OAc), 2.03 (s, 3H, OAc), 2.06 (s, 3H, OAc), 2.12 (s, 3H, OAc), 2.16 (s, 3H, OAc), 2.60 (dd, J = 13.5, 4.9, 1H, H_{3eq}), 3.92 (dd, J = 18.1, 4.6, 1H), 4.05−4.26 (m, 6H), 4.43 (dd, J = 12.5, 2.6, 1H), 5.09 (m, 1H), 5.28−5.33 (m, 2H), 5.38 (br, 1H, N*H*Ac), 7.12 (t, J = 4.5, 1H, N*H*CH_2). ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 170.9, 170.7, 170.3, 170.2, 170.0, 169.2, 167.9, 166.9, 97.6, 72.7, 70.8, 68.2, 67.8, 61.8, 61.5, 49.6, 41.2, 36.9, 23.1, 20.9, 20.8, 20.7, 14.1. IR ($CDCl_3$, cm^{-1}) 3348, 2989, 1745, 1687, 1539, 1371, 1228. HRMS(FAB) calcd for $C_{25}H_{36}N_2O_{15}$: 604.2115, found 627.2046 (M + Na)$^+$. $[\alpha]^{25}_D$ = −0.9 (*c* 1.0, CH_2Cl_2).

Peracetylated NeuAc-Ser-OBn (6): 44% yield. ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.87 (s, 3H, NH*Ac*), 1.88 (m, 1H, H_{3ax}), 2.01 (s, 6H, OAc), 2.06 (s, 3H, OAc), 2.08 (s, 3H, OAc), 2.13 (s, 3H, OAc), 2.60 (dd, J = 12.4, 4.9, 1H, H_{3eq}), 3.15 (br t, 1H), 3.98−4.14 (m, 5H), 4.35 (dd, J = 12.4, 2.5, 1H), 4.49 (m, 1H), 5.11−5.34 (m, 5H), 5.58 (br d, 1H, N*H*Ac), 7.30−7.39 (m, 6H). ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 170.9, 170.9, 170.6, 170.4, 170.1, 169.2, 168.1, 166.8, 135.2, 128.5, 128.4, 128.2, 98.1, 72.8, 70.5, 68.2, 67.7, 67.4, 62.4, 62.0, 55.4, 53..8, 49.4, 36.2, 23.1, 20.9, 20.8, 20.7. IR ($CDCl_3$, cm^{-1}) 3421, 2978, 1741, 1689, 1535, 1359, 1225. HRMS(FAB) calcd for $C_{31}H_{40}N_2O_{16}$: 696.2377, found 697.2469 (M + H)$^+$. $[\alpha]^{25}_D$ = −30.8 (*c* 6.5, CH_2Cl_2).

Peracetylated NeuAc-Ala-OBn (7): 60% yield. ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.47 (d, J = 7.1, 3H, $CHCH_3$), 1.89 (s, 3H, NH*Ac*), 1.98 (m, 1H), 2.02 (s, 6H, OAc), 2.05 (s, 3H, OAc), 2.09 (s, 3H, OAc), 2.17 (s, 3H, OAc), 2.62 (dd, J = 13.3, 4.9, 1H, H_{3eq}), 4.09−4.21 (m, 3H), 4.38 (dd, J = 12.4, 2.6, 1H), 4.54 (m, 1H), 5.13−5.38 (m, 5H), 5.79 (br d, 1H, N*H*Ac), 7.28−7.36 (m, 6H). ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 171.8, 170.8, 170.5, 170.3, 170.1, 169.9, 167.8, 166.1, 128.5, 128.3, 128.1, 97.7, 72.5, 70.4, 68.3, 67.6, 67.1, 61.8, 53.8, 49.4, 48.2, 36.5, 23.0, 20.8, 20.7, 20.6, 17.8. IR ($CDCl_3$, cm^{-1}) 3356, 2968, 2957, 1745, 1691, 1529, 1371, 1228, 1037. HRMS(FAB) calcd for $C_{31}H_{40}N_2O_{15}$: 680.2428, found 681.2510 (M + H)$^+$. $[\alpha]^{25}_D$ = −32.7 (*c* 5.5, CH_2Cl_2).

Peracetylated NeuAc-Gly-OBn (8): 65% yield. ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.89 (s, 3H, NH*Ac*), 1.90 (m, 1H, H_{3ax}), 2.01 (s, 3H, OAc), 2.02 (s, 3H, OAc), 2.05 (s, 3H, OAc), 2.11 (s, 3H, OAc), 2.14 (s, 3H, OAc), 2.58 (dd, J = 14.1, 4.9, 1H, H_{3eq}), 3.97 (dd, J = 18.2, 4.7, 1H), 4.05−4.15 (m, 3H), 4.24 (dd, J = 18.2, 6.3, 1H), 4.42 (dd, J = 12.5, 2.6, 1H), 5.07 (m, 1H), 5.18 (s, 1H), 5.19 (s, 1H), 5.30−5.34 (m, 2H), 5.49 (br d, J = 9.1, 1H, N*H*Ac), 7.15 (t, J = 5.7, 1H, N*H*CH_2), 7.31−7.37 (m, 5H, *Ph*). ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 171.0, 170.7, 170.3, 170.3, 170.1, 169.1, 167.9, 167.0, 135.2, 128.6, 128.5, 128.4, 97.6, 72.8, 70.9, 68.2, 67.9, 67.2, 61.8, 49.7, 41.3, 36.9, 23.2, 20.9, 20.8, 20.8, 20.7. IR ($CDCl_3$, cm^{-1}). HRMS(FAB) calcd for $C_{30}H_{38}$-

(19) Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923.

N_2O_{15}: 666.2271, found 689.2206 $(M + Na)^+$. $[\alpha]^{25}_D = -36.7$ (*c* 1.36 , CH_2Cl_2).

Peracetylated NeuAc-Gly-OH (9): 100% yield. ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.87 (m, 1H), 1.89 (s, 3H, NH*Ac*), 2.02 (s, 3H), 2.05 (s, 3H, OAc), 2.07 (s, 3H, OAc), 2.11 (s, 3H, OAc), 2.13 (s, 3H, OAc), 2.56 (dd, $J = 13.3, 4.8$, 1H, H_{3eq}), 3.93−4.24 (m, 5H), 4.44 (dd, $J = 12.3, 2.4$, 1H), 5.05−5.08 (m, 1H), 5.28−5.35 (m, 2H), 5.87 (br d, $J = 8.8$, 1H, N*H*Ac), 7.23 (t, $J = 5.3$, 1H, N*H*CH_2). ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 171.3, 171.2, 171.2, 171.0, 170.6, 170.2, 168.3, 167.3, 97.5, 72.8, 71.0, 68.3, 67.9, 61.9, 49.5, 41.1, 36.8, 23.0, 20.9, 20.8, 20.7. IR ($CDCl_3$, cm^{-1}) 3334, 2961, 1737, 1676, 1533, 1370, 1228, 1031. HRMS-(FAB) calcd for $C_{23}H_{32}N_2O_{15}$: 576.1802, found 599.1722 $(M + Na)^+$. $[\alpha]^{25}_D = -6.1$ (*c* 6.5, $CDCl_3$).

Methyl 4,7,8,9-tetra-*O*-acetyl-5-*N*-(-Gly-peracetylated-NeuAc)-3,5-dideoxy-2-*O*-methyl-D-glycero-*β*-D-galacto-2-nonulopyranosonate (11): 6% yield. ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.90 (s, 3H, NH*Ac*), 1.95−2.1 (s, OAc's), 2.52 (m, 2H, H_{3eq}), 3.3 (s, 3H, O*Me*), 3.6−3.78 (m, 5H), 3.80 (s, 3H, CO_2*Me*), 3.95−4.4 (m, 9H), 4.95−5.5 (m, 6H), 6.68 (d, 1H, N*H*CO). HRMS(FAB) calcd for $C_{42}H_{59}N_3O_{26}$: 1021.3386, found 1022.3459 $(M + H)^+$.

Peracetylated NeuAc-Gly-Ala-OBn (12): 54% yield. ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.42 (d, $J = 7.2$, 3H, CH*CH*$_3$), 1.83 (m, 1H), 1.88 (s, 3H, N*H*Ac), 2.01 (s, 3H, OAc), 2.02 (s, 6H, OAc), 2.04 (s, 3H, OAc), 2.14 (s, 3H, OAc), 2.64 (dd, $J = 13.0, 5.0$, 1H, H_{3eq}), 3.79 (dd, $J = 16.9, 4.9$, 1H), 4.04−4.13 (m, 3H), 4.23−4.41 (m, 2H), 4.54−4.60 (m, 1H), 5.07-5.09 (m, 1H), 5.13 (s, 2H), 5.33−5.36 (m, 2H), 5.73 (br d, $J = 9.2$, 1H, N*H*Ac), 7.06 (d, $J = 14$, 1H, N*H*CH), 7.13−7.37 (m, 6H). ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 172.1, 171.0, 170.7, 170.5, 170.1, 170.0, 168.9, 168.4, 166.9, 135.6, 129.0, 128.5, 128.2, 127.9, 97.6, 72.9, 70.3, 68.1, 67.7, 66.8, 61.7, 49.4, 48.3, 36.9, 23.1, 20.9, 20.8, 20.7, 17.4. IR (CH_2Cl_2, cm^{-1}) 3381, 1745, 1670, 1534, 1371, 1228. HRMS(FAB) calcd for $C_{31}H_{40}N_2O_{15}$: 680.2428, found 681.2510 $(M + H)^+$. $[\alpha]^{25}_D = -61.5$ (*c* 3.9, CH_2Cl_2).

1,6-Anhydro-3,4-di-*O*-benzyl-2-deoxy-2-*N*-(-Gly-peracetylated-NeuAc)-*β*-D-glucopyranose (15): 58% yield. ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.86 (s, 3H, N*H*Ac), 1.89 (m, 1H), 1.99 (s, 3H, OAc), 2.01 (s, 3H, OAc), 2.03 (s, 3H, OAc), 2.06 (s, 3H, OAc), 2.12 (s, 3H, OAc), 2.60 (dd, $J = 13.5, 4.9$, 1H, H_{3eq}), 3.32 (br s, 1H), 3.47 (br s, 1H), 3.68 (t, $J = 6.7$, 1H), 3.92 (t, $J = 6.0$, 1H), 4.04−4.22 (m, 5H), 4.36−4.52 (m, 5H), 4.72−4.77 (m, 2H), 5.05 (m, 1H), 5.27−5.37 (m, 3H), 5.46 (br d, $J = 9.4$, 1H, N*H*Ac), 6.50 (d, $J = 9.4$, 1H, N*H*CH), 7.20−7.35 (m, 11H). ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 171.171.0, 170.7, 170.4, 170.3, 170.1, 168.3, 167.7, 167.0, 137.9, 137.5, 129.0, 128.6, 128.4, 128.2, 128.1, 127.9, 127.7, 125.3, 100.6, 97.7, 75.7, 74.2, 72.9, 71.7, 71.1, 70.9, 68.2, 68.0, 65.1, 61.8, 49.6, 48.3, 43.0, 36.9, 23.2, 20.9, 20.8, 20.8, 20.8. IR ($CDCl_3$, cm^{-1}) . HRMS(FAB) calcd for $C_{43}H_{53}N_3O_{18}$: 899.3323, found 900.3430 $(M + H)^+$. $[\alpha]^{25}_D = -49.2$ (*c* 7.1, CH_2Cl_2).

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Supporting Information Available: ^{1}H NMR for new compounds **5**-**9**, **11**, **12**, and **15** (8 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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