Synthesis of C-5 Analogs of *N*-Acetylneuraminic Acid via Indium-Mediated Allylation of N-Substituted 2-Amino-2-deoxymannoses

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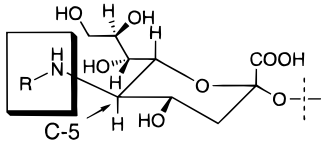
This paper presents a short synthesis of new analogs of *N*-acetylneuraminic acid (Neu5Ac) varied structurally at C-5. The synthetic strategy includes indium-mediated coupling reactions between ethyl 2-(bromomethyl)acrylate and N-derivatized mannosamines, and the ozonolysis of the resulting enoates. The main advantage of this indium-mediated allylation for the synthesis of neuraminic acids comes from the efficient, stereoselective C−C bond formation, which affords predominantly the correct diastereomer having a *threo* relationship between the newly generated hydroxyl group and the C-2 amide group of mannosamine. By this approach, Neu5Boc (**4a**), Neu5Gly (**4b**), Neu5-(6-NHCbz)hexanoyl (**4c**), and Neu5(1-naphthyl)acetyl (**4d**) were prepared in three steps (overall ∼50%). In addition, several N-substituted neuraminic acids were synthesized by *N*-acylation of the amino functionality of neuraminic acid (**5b**), which was obtained by deprotecting the *N*-Boc group of Neu5Boc (**4a**). These analogs include Neu5BrAc (**6a**), Neu5acryloyl (**6b**), Neu5benzoyl (**6c**) and Neu5benzoyl-4-benzoyl (**6d**). The *N*-acylation method is especially suited for synthesis of neuraminic acids bearing substituents that can not tolerate ozonolysis or that are unstable (photo)-chemically. Finally, we illustrate the utility of synthetic neuraminic acids by converting **4c** to a derivative of 2-deoxy-2,3-didehydroneuraminic acid (**8c**), a precursor to inhibitors of neuraminidases.

Introduction

This paper describes an efficient synthesis of a physiologically important carbohydrate, neuraminic acid (Neu),[1] with varying substituents at C-5, and its derivatives related to 2-deoxy-2,3-didehydroneuraminic acid (Neu2en). The crucial step of this approach is the In-mediated, nucleophilic addition of an allylic anion equivalent to N-derivatives of 2-amino-2-deoxymannose (2-mannosamine). The coupling reaction proceeds well in an aqueous medium, and its efficiency has been consistently high among a number of substrates presenting common functional groups such as free OH, *N*-Boc, and *N*-Cbz groups.

A significant number of cellular events are mediated by a variety of carbohydrate-linked proteins and lipid molecules (glycoconjugates).[2] Neuraminic acids or sialic acids refer to a class of structurally unique, natural carbohydrates that occur as a sugar element, terminating in glycoproteins and glycolipids.[3] There are about 30 natural sialic acids, substituted differently at positions C-4 to C-9, including *N*-acetylneuraminic acid (Neu5Ac) as the most ubiquitous analog, *N*-glycolylneuraminic acid (Neu5glycolyl), and other *O*-acetylated analogs (4-*O*-acetylNeu5Ac, 9-*O*-acetylNeu5Ac). Neu5Ac and its analogs are expressed as α-*O*-sialosides on the surface of mammalian cells as a component essential to gangliosides,[2c] glycoconjugates,[2a] and sialyl Lewis x,[4] and also occur as a linear homopolymer (polysialic acid, >50-mer).[5] Carbohydrates of this class mediate numerous cellular recognition events[4] in cell migration and adhesion,[6] immune responses,[7] tumor metastasis,[8] and the development of neural cells.[9] In addition, these cellular moieties constitute a characteristic ligand commonly recognized



R = $COCH_3$;
N-acetylneuraminic acid (**Neu5Ac**)
R = $COCH_2OH$;
N-glycolylneuraminic acid (**Neu5glycolyl**)

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(1) The IUPAC name for neuraminic acid is 5-amino-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulosonic acid.

(2) For review, see: (a) Horowitz, M. I.; Pigman, W. *The Glycoconjugates*; Academic Press: New York, 1977−1985; Vols. 1−4. (b) Karlsson, K.-A. *Curr. Opin. Struct. Biol.* **1995**, *5*, 622. (c) Wiegandt, H. In *Glycolipids*; Wiegandt, H., Ed.; Elsevier: New York, 1985; Vol. 10, p 199. (d) Varki, A. *Glycobiology* **1993**, *3*, 97.

(3) (a) Corfield, A. P.; Schauer, R. In *Sialic Acids, Chemistry, Metabolism and Function*; Schauer, R., Ed.; Springer Verlag: New York, 1982; Vol. 10, pp 5−39. (b) Vliegenthart, J. F. G.; Kamerling, J. P. In *Sialic Acids, Chemistry, Metabolism and Function*; Schauer, R., Ed.; Springer Verlag: New York, 1982; Vol. 10, pp 59−76. (c) Roy, R.; Laferrière, C. A.; Pon, R. A.; Gamian, A. In *Methods in Enzymology (Neoglycoconjugates. Part B. Biomedical Applications)*; Lee, Y. C., Lee, R. T., Eds.; Academic Press, Inc: San Diego, 1994; Vol. 247, pp 351−361.

(4) (a) Magnani, J. L.; Nilsson, B.; Brockhaus, M.; Zopf, D.; Steplewski, Z.; Koprowski, H.; Ginsburg, V. *Cancer Res.* **1983**, *43*, 5489. (b) Danishefsky, S. J.; Koseki, K.; Griffith, D. A.; Gervay, J.; Peterson, J. M.; McDonald, F. E.; Oriyama, T. *J. Am. Chem. Soc.* **1992**, *114*, 8331.

(5) Nakayama, J.; Fukuda, M. N.; Fredette, B.; Ranscht, B.; Fukuda, M. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 7031.

(6) (a) Rossen, S. D.; Bertozzi, C. R. *Curr. Opin. Cell Biol.* **1994**, *6*, 663. (b) Lasky, L. A. *Annu. Rev. Physiol.* **1995**, *57*, 827.

(7) Kelm, S.; Pelz, A.; Schauer, R.; Filbin, M. T.; Tang, S.; Bellard, M.-E. d.; Schnaar, R. L.; Mahoney, J. A.; Hartnell, A.; Bradfield, P.; Crocker, P. R. *Curr. Biol.* **1994**, *4*, 965.

(8) Livingston, B. D.; Jacobs, J. L.; Glick, M. C.; Troy, F. A. *J. Biol. Chem.* **1988**, *263*, 9443.

(9) Edelman, G. M. *Annu. Rev. Biochem.* **1985**, *54*, 135.

(10) Watowich, S. J.; Skehel, J. J.; Wiley, D. C. *Structure* **1994**, *2*, 719.

(11) Stehle, T.; Yan, Y.; Benjamin, T. L.; Harrison, S. C. *Nature* **1994**, *369*, 160.

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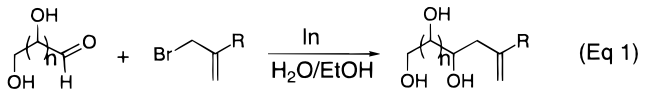
by many infectious pathogens such as viruses (influenza,[10] polyoma,[11] rota,[12] Sendai[13]), bacteria, and parasites.[14] In general, the adsorption of pathogens to target cells, the initial step of infection, results from the binding of pathogenic surface proteins to the sialosides on the cellular surface.[2b,15]

Synthetic analogs of Neu have helped to unravel mechanisms of sialic acid-dependent processes. These analogs have been involved in structure−activity studies,[3b,16] to study the specificities of distinct sialoconjugates in virus infectivity,[17] cellular adhesion,[18] and sialidase resistance.[19] They have been tested also as inhibitors of pathogens.[20] Particularly, analogs of Neu with a fluorescent or photosensitive group at position C-5 (along with C-9)[21] have been used as molecular probes in *in vivo* detection of sialylated glycans,[22] and in enhancing the sensitivity of sialyltransferase assay,[23] and in characterization of new Neu-binding receptors and neuraminidases.[24]

In contrast to the significance and diversity of physiological roles of sialic acids, the use of synthetic Neu has been limited, partially due to a lack of efficient synthetic methods. A number of chemical[25] and chemoenzymatic[26] syntheses of Neu have been developed. These synthetic methods constitute a valuable route to Neu, especially non-natural Neu which is otherwise unavailable by isolation from natural sources.[25c,27] As a methodology important to carbohydrate synthesis, metal (indium, tin, or zinc)-mediated, nucleophilic addition of a carbanion to a carbonyl equivalent adds a useful process that leads to higher carbon sugars (eq 1). This C−C forming, coupling reaction has been applied to nonsugar aldehydes/ketones,[25b,28] aldimines,[29] and aldoses.[25ab,28f,30] In



(Eq 1)

general, this metal-mediated reaction gives high chemical yields, unrestrained reaction scales (milli- to multigram synthesis), and good regio/stereoselectivity. Unlike many organometallic reactions, this reaction is insensitive to the presence of moisture and proceeds well in aqueous media, rendering the protection of the hydroxyl groups of carbohydrate substrates unnecessary.

Recently, Gordon *et al.*[25a] and Chan *et al.*[25b] demonstrated the efficiency of In-mediated reactions by synthesizing per-acetylated or unprotected Neu5Ac starting from a commercially available *N*-acetylmannosamine. The present study extends the scope of In coupling to the synthesis of Neu by demonstrating a general synthesis of C-5 analogs of Neu. We believe that the present method provides a valuable alternative to other synthetic[25,26] and isolation[27] approaches in preparation of Neu, useful in designing syntheses of complex carbohydrates.

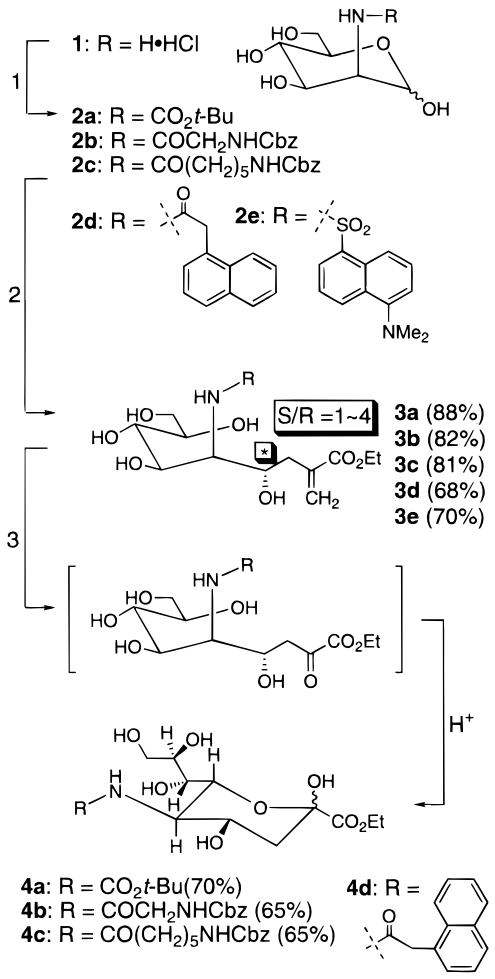
Results and Discussion

Scheme 1 describes a three-step synthesis of *N*-acylated derivatives of Neu (**4a**−**d**; Neu5R). The synthesis began with N-derivatization of 2-mannosamine, which was performed by neutralization of **1** with NaOMe in MeOH, and by treatment with $(Boc)_2O$ or *N*-hydroxysuccinimide esters (*N*-Cbz-glycine, 6-(NH-Cbz)hexanoic acid, and 1-naphthaleneacetic acid). The reaction afforded *N*-Boc or *N*-acylmannosamines (**2a**−**d**) in 85−90% yields. A similar treatment with dansyl chloride afforded *N*-dansylmannosamine (**2e**). Each derivative (**2a**−**d**) consisted of two epimers (C-1) with an average ratio of $\alpha/\beta \sim 3/1$ on the basis of the ^{1}H-NMR spectrum. We proceeded with mixtures of epimers for the next step, because the two isomers are in equilibrium with the same aldehyde, which then reacts with indium reagent.

The In-mediated reaction for C−C bond formation was performed by warming (∼55 °C) a vigorously stirred suspension of indium powder (∼4 equiv), ethyl 2-(bromomethyl)acrylate (∼6 equiv)[31] and unprotected mannosamines (**2a**−**e**) in an acidic medium (0.1 M HCl/EtOH ∼1/7 v/v). The reaction afforded enoates (**3a**−**e**): Scheme 1; R = $COOC(CH_3)_3$ (88%), $COCH_2NHCbz$ (82%), $CO(CH_2)_5NHCbz$ (81%), $COCH_2$-1-$C_{10}H_7$ (68%), SO_2-1-$C_{10}H_6$-5-NMe_2 (70%), $COCH_3$ (90%[25a]). This heterogeneous reaction was run in an acidic medium rather than a

(12) Méndez, E.; Arias, C. F.; López, S. *J. Virol.* **1993**, *67*, 5235.
(13) Dallocchio, F.; tomasi, M.; Bellini, T. *Biochem. Biophys. Res. Commun.* **1995**, *208*, 36.
(14) (a) Stein, P. E.; Boodhoo, A.; Armstrong, G. D.; Heerze, L. D.; Cockle, S. A.; Klein, M. H.; Read, R. J. *Nature Struct. Biol.* **1994**, *1*, 591. (b) Sim, B. K. L.; Chitnis, C. E.; Wasniowska, K.; Hadley, T. J.; Miller, L. H. *Science* **1994**, *264*, 1941.
(15) (a) Lanzrein, M.; Schlegel, A.; Kempf, C. *Biochem. J.* **1994**, *302*, 313. (b) Paulson, J. C. In *The Receptors*; Conn, P. M., Ed.; Academic Press: Orlando, 1985; Vol. 2, pp 131−219. (c) Rossmann, M. G. *Protein Sci.* **1994**, *3*, 1712.
(16) (a) Paulson, J. C.; Rogers, G. N.; Caroll, S. M.; Higa, H. H.; Pritchet, T.; Milks, G.; Sabesan, S. *Pure Appl. Chem.* **1984**, *56*, 797. (b) Brossmer, R.; Gross, H. J. In *Methods in Enzymology (Neoglycoconjugates. Part B. Biomedical applications)*; Lee, Y. C., Lee, R. T., Eds.; Academic Press, Inc.: San Diego, 1994; Vol. 247, pp 153−176.
(17) (a) Pritchett, T. J.; Brossmer, R.; Rose, U.; Paulson, J. C. *Virology* **1987**, *160*, 502. (b) Machytka, D.; Kharitonenkov, I.; Isecke, R.; Hetterich, P.; Brossmer, R.; Klein, R. A.; Klenk, H.-D.; Egge, H. *FEBS Lett.* **1993**, *334*, 117.
(18) Springer, T. A. *Cell* **1994**, *76*, 301.
(19) Sabesan, S.; Neira, S.; Davidson, F.; Duus, J. Ø.; Bock, K. *J. Am. Chem. Soc.* **1994**, *116*, 1616.
(20) (a) Schmid, W.; Avila, L. Z.; Williams, K. W.; Whitesides, G. M. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 747. (b) Murakami, M.; Ikeda, K.; Achiwa, K. *Carbohydr. Res.* **1996**, *280*, 101.
(21) Brossmer, R.; Gross, H. J. In *Methods in Enzymology (Neoglycoconjugates. Part B. Biomedical Applications)*; Lee, Y. C., Lee, R. T., Eds.; Academic Press, Inc.: San Diego, 1994; Vol. 247, pp 177−193.
(22) (a) Gross, H. J.; Rose, U.; Krause, J. M.; Paulson, J. C.; Schmid, K.; Feeney, R. E.; Brossmer, R. *Biochemistry* **1989**, *28*, 7386. (b) Gross, H. J.; Brossmer, R. *Eur. J. Biochem.* **1988**, *177*, 583.
(23) Gross, H. J.; Sticher, U.; Brossmer, R. *Anal. Biochem.* **1990**, *186*, 127.
(24) (a) Horst, G. T. J. v. d.; Mancini, G. M. S.; Brossmer, R.; Rose, U.; Verheijen, F. W. *J. Biol. Chem.* **1990**, *265*, 10801. (b) Mirelis, P.; Brossmer, R. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 2809.
(25) (a) Gordon, D. M.; Whitesides, G. M. *J. Org. Chem.* **1993**, *58*, 7937. (b) Chan, T.-H.; Lee, M.-C. *J. Org. Chem.* **1995**, *60*, 4228. (c) Roy, R.; Laferriere, C. A. *Can. J. Chem.* **1990**, *68*, 2045. (d) Paulsen, H. *Angew. Chem., Int. Ed. Engl.* **1990**, *29*, 823.
(26) (a) For review, see: Gijsen, H. J. M.; Qiao, L.; Fitz, W.; Wong, C.-H. *Chem. Rev.* **1996**, *96*, 443. (b) Lin, C.-H.; Sugai, T.; Halcomb, R. L.; Ichikawa, Y.; Wong, C.-H. *J. Am. Chem. Soc.* **1992**, *114*, 10138. (c) Simon, E. S.; Bednarski, M. D.; Whitesides, G. M. *J. Am. Chem. Soc.* **1988**, *110*, 7159. (d) Schrell, A.; Whitesides, G. M. *Liebigs Ann. Chem.* **1990**, 1111. (e) Bednarski, M. D.; Waldmann, H. J.; Whitesides, G. M. *Tetrahedron Lett.* **1986**, *27*, 5807. (f) Augé, C.; David, S.; Veyrieres, C. G. e. A. *Tetrahedron Lett.* **1985**, *26*, 2439.
(27) Czarniecki, M. F.; Thornton, E. R. *J. Am. Chem. Soc.* **1977**, *99*, 8273.
(28) (a) Chao, L. C.; Rieke, R. D. *J. Org. Chem.* **1975**, *40*, 2253. (b) Petrier, C.; Einhorn, J.; Luche, J. L. *Tetrahedron Lett.* **1985**, *26*, 1449. (c) Araki, S.; Ito, H.; Butsugan, Y. *J. Org. Chem.* **1988**, *53*, 1831. (d) Li, C. J.; Chan, T. H. *Tetrahedron Lett.* **1991**, *32*, 7017. (e) Paquette, L. A.; Lobben, P. C. *J. Am. Chem. Soc.* **1996**, *118*, 1917. (f) Paquette, L. A.; Mitzel, T. M. *J. Am. Chem. Soc.* **1996**, *118*, 1931.
(29) Araki, S.; Katsumura, N.; Ito, H.; Butsugan, Y. *Tetrahedron Lett.* **1989**, *30*, 1581.
(30) (a) Schmid, W.; Whitesides, G. M. *J. Am. Chem. Soc.* **1991**, *113*, 6674. (b) Kim, E.; Gordon, D. M.; Schmid, W.; Whitesides, G. M. *J. Org. Chem.* **1993**, *58*, 5500. (c) Chan, T. H.; Li, C.-J. *J. Chem. Soc., Chem. Commun.* **1992**, 747. (d) Gao, J.; Härter, R.; Gordon, D. M.; Whitesides, G. M. *J. Org. Chem.* **1994**, *59*, 3714.
(31) Villieras, J.; Rambaud, M. *Org. Synth.* **1988**, *66*, 220.

Scheme 1[a]



[a] (1) NaOMe, $(Boc)_2O$, or X-COR, MeOH, rt, 24 h; (2) In, ethyl α-bromomethacrylate, EtOH, 0.1 M HCl, 55 °C, 36 h; (3) O_3, MeOH, −78 °C, 30 min; then HCO_2H, H_2O_2, H_2O, rt, 90 min.

neutral solvent, because the presence of a small amount of HCl increased the rate and efficiency of the coupling reaction.[25a,30b] The scale of the reaction could be varied within a laboratory scale (0.1 mg to ≥5 g of mannosamines) without noticeable change in yield.

The success of this reaction is ascribed to the selective reactivity of indium toward allylic halides and to its inability to reduce the aldehyde function.[28,29,32] Clearly, the products resulted from a nucleophilic addition of an allylic anion equivalent (as a ligand chelated to In[28c]) to an aldehyde of **2**. Each addition reaction resulted in formation of a mixture composed of two diastereomers **3** (threo/erythro or *S*/*R*) at the newly generated carbon center (marked *). In most cases, the major isomer could not be cleanly separated by flash column chromatography (silica gel; 5−20% MeOH/CH_2Cl_2), but its purity could be increased to ≥90% after repeated (≥2) chromatography.

The stereochemistry at the chiral center of the major isomer was determined unambiguously as *S* after ozonolysis (Scheme 1: O_3, MeOH, −78 °C) of **3**. Compound **4** (R = $COCH_3$; Neu5Ac), obtained by this method, was indistinguishable from authentic Neu5Ac[25c,27] isolated from edible bird nests, with respect to ^{1}H- and ^{13}C-NMR spectroscopy, mass spectrometry, and thin layer chromatography.[25a] Here, the chemical shifts and coupling constants of the two C-3 protons (H_{ax} and H_{eq}) of Neu are influenced characteristically by the stereochemistry of the hydroxyl group substituted at C-4.[27,33]

We estimated the ratio (threo/erythro or *S*/*R*) between the two diastereomers (**3**) by integration of distinct olefinic signals in the ^{1}H-NMR spectrum. For substrates **2a**−**c**, the *S*/*R* ratio was 3.5−4.0, but with **2d** and **2e**, the selectivity was low, ∼1.5 and ∼1.0, respectively. The preferential formation of the *threo* product was observed also in other indium (or tin)-mediated allylations with aldoses bearing a C-2 hydroxyl group.[28d,f,30a,b,d] The molecular basis of the stereochemical outcome of this reaction (from N-substituted mannosamines) has not been clearly established, but a model was proposed on the basis of Cram-type chelation.[25a,28f] This mechanism suggests a formation of a five-membered chelate between In(R) and an aldose (via coordination of the metal to aldehyde oxygen and amide nitrogen), and the attack of an allylic nucleophile to an aldehyde of this complex, preferentially from the less-hindered side.[25a,28f] According to this model, the structural and functional properties of N-substituents should influence the efficiency of formation of the chelate as well as the approach of a nucleophile. We believe that the low selectivity observed from **2d** (naphthalenylacetyl) and **2e** (*N*-sulfonamide) may be related to these factors.

The efficiency of ozonolysis of the enoates was almost quantitative from ^{1}H-NMR spectroscopy, but the yields of **4** (Neu5R), after purification of the crude product by chromatography (silica gel, 10% MeOH/CH_2Cl_2 to 5% HCOOH/30% MeOH/CH_2Cl_2), were ∼65−70% (**4a**−**c**) and 27% (**4d**). It is noteworthy that unprotected **4** was isolated directly, without having to convert it to the peracetylated Neu5R.[25a] The direct isolation might have reduced the yield due to incomplete chromatographic recovery of polar Neu5R adsorbed to silica gel. Additionally, certain functional groups (aromatic, dansyl) underwent partial or complete decomposition under the ozonolysis conditions. This decomposition was observed for enoates **3d** (27% for **4d**) and **3e** (0%). Accordingly, the present method is limited to enoates with N-substituents stable to ozonolysis.

Derivatives of Neu that were unsuitable for preparation by Scheme 1 could be prepared by N-derivatization of the amino group of Neu. There are several methods, either chemical[25c] or chemoenzymatic,[26d,34] for synthesis of *N*-deacetylated Neu. Scheme 2 summarizes an alternative approach for the synthesis of Neu and its *N*-acylation, complementary to previous methods. The treatment of **4a** with TFA/CH_2Cl_2 (or 1.0 M HCl/MeOH) removed the *N*-Boc group quantitatively, but afforded undesired product(s), believed to be imines. Therefore, we acetylated **4a** exhaustively by use of Ac_2O in pyridine and then deprotected the *N*-Boc group of **5a**. Product **5b** was allowed to react with acid chlorides in the presence of *i*-Pr_2NEt as base. This *N*-acylation afforded Neu5R (**6a**−**d**; 36−60%) containing *N*-$COCH_2Br$, $COCH{=}CH_2$, or COC_6H_4-4-COC_6H_5); these species can not be prepared according to Scheme 1.

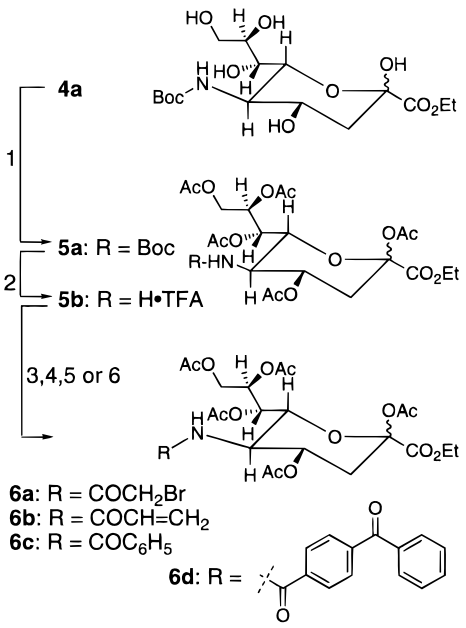
The methods described in Schemes 1 and 2 represent a convenient route to various C-5 analogs of Neu, such

(32) Beuchet, P.; Marrec, N. L.; Mosset, P. *Tetrahedron Lett.* **1992**, *33*, 5959.

(33) Schreiner, E.; Zbiral, E.; Kleineidam, R. G.; Schauer, R. *Liebigs Ann. Chem.* **1991**, 129.

(34) Sparks, M. A.; Williams, K. W.; Lukacs, C.; Schrell, A.; Priebe, G.; Spaltenstein, A.; Whitesides, G. M. *Tetrahedron* **1993**, *49*, 1.

Scheme 2[a]



[a] (1) Ac_2O, pyridine, 69%; (2) TFA, CH_2Cl_2, rt, quantitative; (3) $BrCH_2COBr$, *i*-Pr_2NEt, CH_2Cl_2, rt, 12 h, 60%; (4) CH_2=CHCOCl, *i*-Pr_2NEt, CH_2Cl_2, 36%; (5) C_6H_5COCl, *i*-Pr_2NEt, CH_2Cl_2, 58%; (6) $C_6H_5COC_6H_4COCl$, *i*-Pr_2NEt, CH_2Cl_2, 60%.

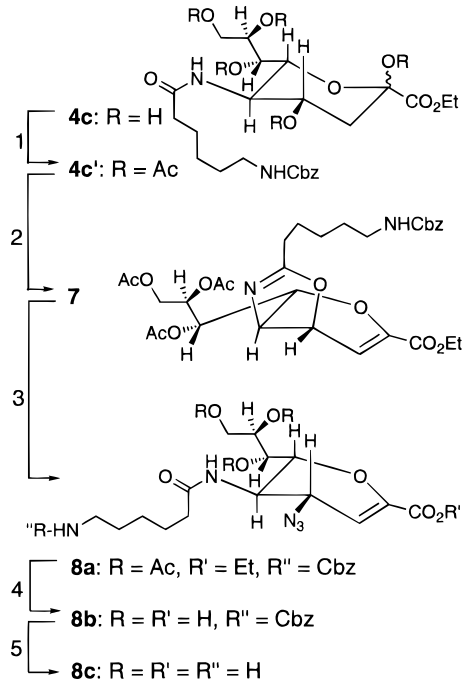
as amino acid conjugates (**4b**, **4c**), and (photo)chemically cross-linkable (**6a**, **6b**, **6d**) analogs. These functional Neu5R may be potentially useful as (photo)affinity-labeled or fluorescent Neu in photolabeling studies[21−24,35] of sialic acid-dependent cellular processes.

Scheme 3 summarizes a synthesis of analogs of 2-deoxy-2,3-dehydroneuraminic acid (Neu2en).[33,36] Neu2en belongs to a class of inhibitors of neuraminidase,[37] an enzyme that catalyzes the hydrolytic cleavage of C(2)−O bond of α-*O*-sialosides. Sialic acid **4c** was acetylated exhaustively. Product **4c′** was converted to oxazole **7** by treatment with TMSOTf according to literature methods.[33,38] This oxazole was transformed to Neu2en **8a** by treatment with $TMSN_3$, which resulted in ring-opening and the substitution of azide nucleophile at position C-4 with an inversion of stereochemistry.[33,38] The hydrolysis and deprotection of *N*-Cbz group led to unprotected Neu2en **8c**. This example illustrates the use of a synthetic Neu as a precursor to an analog of azido-substituted dehydroneuraminic acid. Additionally, the analog contains an ϵ-amino linker group tethered to C-5; this linker may be useful in tethering an additional probe for studies of the active site of neuraminidases.[24a]

Conclusions

We presented a short synthesis of analogs of (dehydro)-neuraminic acids. The synthesis relies on the previously

Scheme 3[a]



[a] (1) Ac_2O, pyridine, 48 h, 72%; (2) $CF_3SO_3SiMe_3$, MeCN, 50 °C, 2.5 h, 73%; (3) Me_3SiN_3, *t*-BuOH, 80 °C,4 h, 81%; (4) LiOH, MeOH, H_2O, rt, 12 h, 62%; (5) CF_3SO_3H, TFA, 0 °C, 30 min, 33%.

disclosed, In-mediated allylation of unprotected carbohydrates in aqueous ethanol.[25a,30b] The strategy is limited slightly by the cost of indium and 2-amino-2-deoxymannose, but its efficiency is comparable to that of the (chemo)enzymatic syntheses of sialic acids[26a,39] or its isolation from bird nests.[25c,27] Particularly, the present method is a versatile means of introducing various chemical functionalities such as amino acids, (photo)-affinity-labels or fluorescent tags to position C-5 of neuraminic acid.

Experimental Section

General. Tetrahydrofuran (THF) was distilled from sodium benzophenone ketyl. Methylene chloride (CH_2Cl_2) was distilled after refluxing over CaH_2. Indium (In) metal (99.99%; Aldrich) was used as received in powder form. Thin layer chromatography (TLC) was performed on silica gel precoated glass plates (E. Merck, Darmstadt). Flash column chromatography was performed on silica gel 60_{F254} (230−400 mesh, E. Merck). Ozonolysis was performed with an ozone generator (Griffin Technics Inc).

Typical Procedure for N-Derivatization of 2-Amino-2-deoxymannose Hydrochloride. To a solution of 2-amino-2-deoxy-D-mannopyranose hydrochloride (**1**) (2.0 g, 9.3 mmol) and NaOMe (0.56 g, 10.4 mmol) in 80 mL of MeOH were added *N*-Cbz-glycine *N*-hydroxysuccinimide ester (3.4 g, 11.1 mmol) and Et_3N (1.3 mL, 9.3 mmol). After stirring for 30 h at rt, the reaction mixture was concentrated *in vacuo* to yield a pale yellow oil. This thick oily residue was flash chromatographed on silica gel (300 g) by eluting with 5% MeOH/CH_2Cl_2 and then 20% MeOH/CH_2Cl_2. The desired product **2b**, containing a mixture of two epimers (α/β), was obtained as a light yellow, semicrystalline oil (3.1 g, 90%). R_f = 0.5 (20% MeOH/CH_2Cl_2). ^{1}H-NMR (400.14 MHz, CD_3OD): δ (ppm) 7.35−7.27 (m, 5H), 5.05 (s, 2H), 4.44 (d, 0.3H, J = 4.2 Hz), 4.32−4.30 (d, 0.7H, J = 4.5 Hz), 4.04−4.0 (dd, 1H, J = 4.6, 9.7 Hz), 3.88 (s, 2H), 3.86−3.68 (m, 4H), 3.62−3.57 (t, 0.7H, J = 9.5 Hz), 3.50−3.46 (t, 0.3H, J = 9.5 Hz); ^{13}C-NMR (100.61 MHz, CD_3OD): δ (ppm)

(35) (a) Gross, H. J.; Bünsch, A.; Paulson, J. C.; Brossmer, R. *Eur. J. Biochem.* **1987**, *168*, 595. (b) Warner, T. G. *Biochem. Biophys. Res. Commun.* **1987**, *148*, 1323. (c) Warner, T. G.; Lee, L. A. *Carbohydr. Res.* **1988**, *176*, 211.

(36) (a) Itzstein, M. v.; Wu, W.-Y.; 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.; Ryan, D. M.; Cameron, J. M.; Penn, C. R. *Nature* **1993**, *363*, 418. (b) Zbiral, E.; Schreiner, E.; Christian, R.; Kleineidam, R. G.; Schauer, R. *Liebigs Ann. Chem.* **1989**, 159.

(37) (a) Colman, P. M. *Protein Sci.* **1994**, *3*, 1687. (b) Air, G. M.; Laver, W. G. *Proteins* **1989**, *6*, 341.

(38) Itzstein, M. v.; Wu, W.-Y.; Jin, B. *Carbohydr. Res.* **1994**, *259*, 301.

(39) For review, see: DeNinno, M. P. *Synthesis* **1991**, 583.

177.09, 172.38, 137.73, 129.20, 128.74, 128.53, 94.38, 74.07, 70.40, 68.33, 67.48, 62.00, 54.79, 47.50, 44.47, 25.87, 8.90; FAB-MS (NBA/NaI): *m/z* 393 $[M + Na]^+$; HRMS: calcd for $C_{16}H_{22}N_2O_8Na$ 393.1272, found 393.1274.

2a: 89%; R_f = 0.6 (30% MeOH/CH_2Cl_2); ^{1}H-NMR (250.13 MHz, CD_3OD): δ (ppm) 5.01 (s, 1H), 4.00−3.92 (m, 2H), 3.85−3.69 (m, 2H), 3.62−3.35 (m, 2H), 1.44 (s, 9H); ^{13}C-NMR (101.61 MHz, CD_3OD): δ (ppm) 95.30, 95.10, 80.36, 78.24, 74.52, 73.43, 70.64, 68.41, 68.11, 62.23, 62.12, 56.33, 28.73; FAB-MS (NBA/NaI): *m/z* 302 $[M + Na]^+$; HRMS: calcd for $C_{11}H_{21}NO_7Na$ 302.1214, found 302.1216.

2c: 89%; R_f = 0.6 (20% MeOH/CH_2Cl_2); ^{1}H-NMR (300.14 MHz, CD_3OD): δ (ppm) 7.34−7.27 (m, 5H), 5.05 (s, 2H), 4.99 (d, $H_{1\alpha}$, J = 1.4 Hz), 4.38 (dd, $H_{2\beta}$), 4.29−4.27 (dd, $H_{2\alpha}$, J = 1.4, 4.7 Hz), 4.03−3.98 (dd, $H_{3\alpha}$, J = 4.7, 9.6 Hz), 3.85−3.73 (m, 3H), 3.62−3.55 (t, $H_{4\alpha}$, J = 9.6 Hz), 3.53−3.48 (t, 2H, J = 6.8 Hz), 2.33−2.24 (quin, 1.5H, J = 7.3 Hz), 2.18−2.16 (t, 0.5H, J = 7.2 Hz), 1.65−1.57 (quin, 2H, J = 7.3 Hz), 1.52−1.45 (quin, 2H, J = 7.2 Hz), 1.40−1.32 (quin, 2H, J = 7.3 Hz); ^{13}C-NMR (100.61 MHz, CD_3OD): δ (ppm) 176.54, 158.48, 138.34, 129.47, 129.36, 128.79, 128.50, 94.68, 73.40, 70.55, 68.43, 66.96, 62.18, 54.79, 41.49, 36.60, 30.42, 27.30, 26.40; FAB-MS (NBA/NaI): *m/z* 449 $[M + Na]^+$; HRMS: calcd for $C_{20}H_{30}N_2O_8Na_1$ 449.1898, found 449.1900.

2d: 85%; R_f = 0.72 (30% MeOH/CH_2Cl_2); ^{1}H-NMR (250 MHz, CD_3OD): δ (ppm) 8.08−8.01 (br d, 1H, J = 15.4 Hz), 7.87−7.84 (dd, 1H, J = 2.0, 8.1 Hz), 7.79−7.76 (dd, 1H, J = 2.0, 7.4 Hz), 7.55−7.38 (m, 4H), 5.04 (s, 1H), 4.44 (br d, 0.3 H, J = 4.6 Hz), 4.31−4.29 (dd, 0.7 H, J = 1.3, 4.6 Hz), 4.13−4.08 (d, 1H, J = 11.3 Hz), 4.05−4.0 (dd, 1H, J = 4.6, 9.6 Hz), 3.82−3.78 (m, 2H), 3.62−3.58 (m, 1H), 2.65−2.61 (m, 2H); ^{13}C-NMR (100.55 MHz, CD_3OD): δ (ppm) 175.07, 174.55, 171.38, 135.29, 133.67, 133.61, 129.61, 128.84, 128.75, 127.24, 126.75, 126.54, 125.0, 94.79, 73.47, 70.56, 62.38, 55.31, 47.82, 29.92, 26.24; FAB-MS (NBA/NaI): *m/z* 370 $[M + Na]^+$; HRMS: calcd for $C_{18}H_{21}NO_6Na$ 370.1265, found 370.1266.

Typical Procedure of In-Mediated Allylation.[25a,b] To a solution of **2b** (2.3 g, 6.22 mmol) and ethyl 2-(bromomethyl)-acrylate[31] (7.2 g, 37.3 mmol) in 60 mL of EtOH and 9 mL of 0.1 M HCl was added slowly indium powder (3.2 g, 27.9 mmol) at rt. After stirring for 10 min, the mixture was gradually heated to 55 °C and stirred vigorously for 2 d at the same temperature. At the conclusion of the reaction, the mixture was divided into six plastic centrifuge bottles (15 mL) and spun (2000 rpm) for 1 h, after which a homogeneous solution was separated from the indium reagent (white paste). The clear supernatants were combined and concentrated to a colorless oil. The residue was chromatographed on silica gel (400 g) by eluting with 5% MeOH/CH_2Cl_2 and then 20% MeOH/CH_2Cl_2. The desired product **3b** (R = $COCH_2NHCbz$) was obtained as a colorless oil in 82% (*S*/*R* ∼4/1) yield (2.47 g). R_f = 0.76 (20% MeOH/CH_2Cl_2). ^{1}H-NMR (250.13 MHz, CD_3OD): δ (ppm) 7.36−7.28 (m, 5H), 6.20 (br s, 1H), 5.68 (br s, 1H), 5.12 (s, 2H), 4.36−4.28 (m, 1H), 4.19−4.13 (q, 2H, J = 7.0 Hz), 3.84 (s, 2H), 3.88−3.65 (m, 5H), 2.52−2.38 (m, 2H), 1.34−1.24 (t, 3H, J = 7.0 Hz); ^{13}C-NMR (100.55 MHz, CD_3OD): δ (ppm) 173.66, 168.52, 137.99, 129.35, 128.91, 128.81, 128.60, 73.15, 71.09, 69.32, 68.61, 63.96, 61.98, 54.89, 49.84, 37.83, 14.43, 9.33; FAB-MS (NBA/NaI): *m/z* 507 $[M + Na]^+$; HRMS: calcd for $C_{22}H_{32}N_2O_{10}Na$ 507.1953, found 507.1955.

3a (R = $COOC(CH_3)_3$): 88% (*S*/*R* ∼ 3.5/1). R_f = 0.87 (30% MeOH/CH_2Cl_2). ^{1}H-NMR (250.13 MHz, CD_3OD): δ (ppm) 6.22−6.21 (d, 1H, J = 1.5 Hz), 5.68 (d, 1H, J = 1.5 Hz), 4.31−4.26 (t, 1H, J = 6.5 Hz), 4.23−4.15 (q, 2H, J = 7.1 Hz), 3.98−3.52 (m, 6H), 2.50−2.47 (br d, 2H), 1.44 (s, 9H), 1.32−1.26 (t, 3H, J = 7.1 Hz); ^{13}C-NMR (100.55 MHz, CD_3OD): δ (ppm) 168.57, 158.89, 138.25, 136.17, 95.05, 80.93, 73.19, 70.58, 68.25, 64.16, 61.96, 37.83, 28.64; FAB-MS (NBA/NaI): *m/z* 416 $[M + Na]^+$; HRMS: calcd for $C_{17}H_{31}NO_9Na$ 416.1895, found 416.1897.

3c (R = $CO(CH_2)_5NHCbz$): 81% (*S*/*R* ∼ 4/1). R_f = 0.73 (20% MeOH/CH_2Cl_2). ^{1}H-NMR (300.14 MHz, CD_3OD): δ (ppm) 7.33−7.27 (m, 5H), 6.21 (d, 1H, J = 1.3 Hz), 5.69 (br s, 1H), 5.04 (s, 2H), 4.31−4.28 (t, 1H, J = 6.4 Hz), 4.19−4.15 (q, 2H, J = 7.4 Hz), 3.96−3.94 (d, 1H, J = 9.8 Hz), 3.89−3.87 (d, 1H, J = 9.8 Hz), 3.83−3.81 (m, 1H), 3.79−3.75 (m, 2H), 3.61−3.58 (dd, 1H, J = 5.8, 12.5 Hz), 3.41−3.39 (d, 1H, J = 8.4 Hz), 3.13−3.09 (t, 2H, J = 6.8 Hz), 2.52−2.47 (dd, 1H, J = 7.9 Hz), 2.44−2.40 (dd, 1H, J = 5.3 Hz), 2.35−2.32 (m, 2H), 1.67−1.60 (m, 2H), 1.30−1.25 (t, 3H, J = 7.1 Hz); ^{13}C-NMR (100.61 MHz, CD_3OD): δ (ppm) 177.31, 168.57, 129.40, 128.89, 128.72, 128.18, 72.52, 72.05, 69.67, 67.27, 65.18, 61.90, 54.66, 41.61, 38.10, 36.87, 30.58, 27.42, 14.48; FAB-MS (NBA/NaI): *m/z* 563 $[M + Na]^+$; HRMS: calcd for $C_{26}H_{40}N_2O_{10}Na$ 563.2578, found 563.2581.

3d (R = $COCH_2$-1-$C_{10}H_7$): 68% (*S*/*R* ∼ 1.5/1). R_f = 0.72 (20% MeOH/CH_2Cl_2). ^{1}H-NMR (300.14 MHz, CD_3OD): δ (ppm) 8.05−7.95 (m, 2H), 7.86−7.73 (m, 2H), 7.54−7.37 (m, 3H), 6.07−6.04 (two s, 1H), 5.50−5.32 (two s, 1H), 4.10−4.04 (q, 2H, J = 7.9 Hz), 3.85−3.45 (m, 6H), 2.59−2.48 (m, 2H), 1.29−1.26 (t, 3H, J = 7.9 Hz); FAB-MS (NBA/NaI): *m/z* 484 $[M + Na]^+$; HRMS: calcd for $C_{24}H_{31}NO_8Na$ 484.1946, found 484.1947.

3e (R = SO_2-1-$C_{10}H_6$-5-NMe_2): 80% (*S*/*R* ∼ 1/1). ^{1}H-NMR (399.88 MHz, CD_3OD): δ (ppm) 8.54−8.52 (d, 1H, J = 7.6 Hz), 8.42−8.3 (m, 1H), 8.23−8.20 (m, 1H), 7.59−7.45 (m, 2H), 7.29−7.23 (m, 1H), 5.60 and 5.85 (d, 1H, J = 1.6 Hz), 5.29 (d, 1H, J = 1.6 Hz), 4.07−4.03 (q, 2H, J = 7.0 Hz), 3.92−3.85 (m, 1H), 3.81−3.31 (m, 6H), 2.86 (two S, 6H), 2.44−2.39 (dd, 0.5H, J = 3.0, 14.1 Hz), 2.08−2.02 (dd, 0.5H, J = 9.7, 14.1 Hz), 1.71−1.66 (dd, 0.5H, J = 3.0, 14.1 Hz), 1.58−1.52 (dd, 0.5H, J = 9.7, 14.1 Hz), 1.22−1.17 (t, 3H, J = 7.0 Hz); ^{13}C-NMR (100.55 MHz, CD_3OD): δ (ppm) 168.33, 153.41/153.30, 139.0/138.73, 137.87/137.55, 131.14/130.89, 129.48/129.24, 128.40, 127.72/127.35, 124.33, 120.57, 116.45, 116.32, 115.65, 94.63, 73.30, 72.28, 71.16, 70.05, 69.18, 65.34, 62.69, 60.53, 59.29, 37.26, 14.44; FAB-MS (NBA/NaI): *m/z* 549 $[M + Na]^+$, 526 $[M]^+$; HRMS: calcd for $C_{24}H_{34}N_2O_9SNa$ 549.1881, found 549.1883.

Typical Procedure for Ozonolysis. Compound **3b** (R = $COCH_2NHCbz$; 1.1 g, 2.27 mmol) was solubilized in 50 mL of MeOH, after which the solution was cooled to −78 °C. Ozone gas was bubbled into the methanolic solution while stirring it at −78 °C. After 30 min, the ozone bubbling was stopped, and a mixture of H_2O (10 mL), 30% H_2O_2 (5 mL), and HCO_2H (2 mL) was added to the solution. The mixture was gradually warmed to rt while stirring it for 90 min under air. The clear solution was concentrated *in vacuo* to yield a pale yellow foam. The crude product was purified by flash column chromatography on silica gel (50 g) by eluting with 10% MeOH/CH_2Cl_2 and then 5% HCO_2H/30% MeOH/CH_2Cl_2. The product **4b** was obtained as a white foam (0.72 g, 65%). R_f = 0.76 (20% MeOH/CH_2Cl_2). ^{1}H-NMR (399.88 MHz, CD_3OD): δ (ppm) 7.38−7.29 (m, 5H), 5.11 (s, 2H), 4.38−4.32 (q, 2H, J = 7.1 Hz), 4.17−4.10 (m, 2H), 3.96−3.78 (m, 4H), 3.88 (s, 2H), 3.73−3.70 (t, 1H, J = 6.6 Hz), 2.33−2.29 (dd, 1H, J = 4.9, 12.7 Hz), 1.78−1.72 (t, 1H, J = 12.7 Hz), 1.36−1.32 (t, 3H, J = 7.1 Hz); ^{13}C-NMR (100.55 MHz, CD_3OD): δ (ppm) 174.18, 173.77, 159.70, 137.85, 129.45, 129.04, 128.84, 96.96, 73.35, 72.07, 69.20, 67.97, 67.08, 64.61, 63.81, 57.87, 45.15, 40.89, 14.22; FAB-MS (NBA/NaI): *m/z* 509 $[M + Na]^+$; HRMS: calcd for $C_{21}H_{30}N_2O_{11}Na$ 509.1745, found 509.1747.

4a: White foam. 70%. ^{1}H-NMR (300.14 MHz, CD_3OD): δ (ppm) 4.31−4.22 (q, 2H, J = 7.1 Hz), 4.02−3.52 (m, 7H), 2.29−2.23 (dd, 1H, J = 4.9, 12.3 Hz), 1.87−1.79 (t, 1H, J = 12.3 Hz), 1.43 (s, 9H), 1.33−1.28 (t, 3H, J = 7.1 Hz); FAB-MS (NBA/NaI): *m/z* 418 $[M + Na]^+$; HRMS: calcd for $C_{16}H_{29}NO_{10}Na$ 418.1687, found 418.1689.

4c: White foam. 65%. R_f = 0.68 (20% MeOH/CH_2Cl_2). ^{1}H-NMR (300.14 MHz, CD_3OD): δ (ppm) 7.33−7.27 (m, 5H), 5.05 (s, 2H), 4.27−4.22 (q, 2H, J = 6.9 Hz), 4.13−4.09 (m, 1H, H_4), 4.03−4.01 (dd, 1H, J = 10.3 Hz, H_6), 3.85−3.81 (t, 1H, J = 10.2, H_5), 3.77−3.75 (dd, 1H, $J_{8,9'}$ = 3.5 Hz, $J_{9,9'}$ = 8.6 Hz, H_9), 3.67−3.64 (m, 1H, H_8), 3.60−3.58 (dd, 1H, J = 5.8 Hz, $H_{9'}$), 3.58−3.56 (dd, 1H, $J_{7,8}$ = 6.9 Hz, H_7), 3.12−3.09 (t, 2H, J = 6.9 Hz), 2.29−2.27 (t, 2H, J = 7.5 Hz), 2.26−2.23 (dd, 1H, $J_{3eq,4}$ = 4.9 Hz, $J_{3eq,3ax}$ = 11.4 Hz, H_{3eq}), 1.86−1.81 (t, 1H, J = 11.4 Hz, H_{3ax}), 1.67−1.62 (quin, 2H), 1.52−1.47 (quin, 2H), 1.37−1.34 (m, 2H), 1.33−1.28 (t, 3H, J = 6.9 Hz); ^{13}C-NMR (100.55 MHz, CD_3OD): δ (ppm) 192.05, 178.11, 171.76, 129.43, 128.92, 128.71, 96.56, 72.07, 71.66, 70.12, 67.66, 67.28, 64.69, 63.14, 54.09, 41.58, 40.79, 40.15, 36.93, 30.50, 27.29, 26.64, 14.31;

FAB-MS (NBA/NaI): m/z 565 $[M + Na]^+$, 543 $[M + H]^+$; HRMS: calcd for $C_{25}H_{38}N_2O_{11}Na$ 565.2371, found 565.2373.

4d: Pale brown oil. 27%. $R_f = 0.47$ (15% MeOH/CH_2Cl_2). ^{1}H-NMR (300.14 MHz, CD_3OD): δ (ppm) 8.23−8.09 (m, 2H), 7.85−7.73 (m, 2H), 7.64−7.35 (m, 3H), 4.32−4.20 (q, 2H, J = 7.1 Hz), 4.18−4.04 (m, 4H), 3.99−3.62 (m, 5H), 2.34−2.28 (dd, 1H, J = 4.87, 12.87 Hz, H_{3eq}), 1.83−1.75 (t, 1H, J = ∼12.3 Hz, H_{3ax}), 1.33−1.26 (t, 3H, J = 7.1 Hz); FAB-MS (glycerol): m/z 464 $[M + H]^+$.

Acetylation of 4a. Ac_2O (20 mL) was added to **4a** (3.0 g, 7.59 mmol) in 20 mL of pyridine. After 48 h stirring at rt, the volatile solvents were removed by evaporation. The pale red oil was purified with flash silica gel (200 g) chromatography by eluting with 10% MeOH/CH_2Cl_2. The product **5a** was obtained as a pale yellow oil in 69% yield (3.17 g). ^{1}H-NMR (250.13 MHz, CD_3OD): δ (ppm) 5.47−5.43 (dd, 1H, J = 2.4, 4.0 Hz), 5.14−5.04 (m, 2H), 4.48−4.42 (dd, 1H, J = 2.6, 12.4 Hz), 4.27−4.16 (q, 2H, J = 7.1 Hz), 4.12−4.05 (dd, 1H, J = 6.1, 12.4 Hz), 3.77−3.62 (m, 1H), 2.55−2.48 (m, 1H), 2.11−1.95 (multiple s, 15H), 1.30−1.24 (t, 3H, J = 7.1 Hz); FAB-MS (NBA/NaI): m/z 628 $[M + Na]^+$; HRMS: calcd for $C_{26}H_{39}NO_{15}Na$ 628.2215, found 628.2217.

Deprotection of *N*-Boc of 5a. To compound **5a** (1.9 g, 3.15 mmol) dissolved in 20 mL of anhyd CH_2Cl_2 was added TFA (10 mL) at 0 °C. After stirring for 2 h at the same temperature and evaporation of the mixture, a pale red oil was obtained. The residue was solubilized in 10 mL of MeOH, followed by evaporation: this procedure was repeated five times to remove free TFA. The crude product **5b** was dried *in vacuo* and used in the next step without further purification. $R_f = 0.84$ (20% MeOH/CH_2Cl_2). ^{1}H-NMR (399.88 MHz, CD_3OD): δ (ppm) 5.42−5.40 (br d, 1H, J = 8.5 Hz), 5.31−5.2 (m, 2H), 4.47−4.46 (br d, 1H, J = 12.7 Hz), 4.32−4.12 (m, 3H), 4.20−4.18 (q, 2H, J = 7.1 Hz), 2.65−2.61 (m, 1H, H_{3eq}), 2.2−2.0 (m, 16H), 1.26−1.23 (t, 3H, J = 7.1 Hz); ^{13}C-NMR (100.55 MHz, CD_3OD): δ (ppm) 173.74, 171.65, 170.91, 169.78, 166.96, 98.09, 71.25, 70.43, 69.43, 68.56, 67.50, 63.62, 50.96, 35.95, 20.94−20.55 (m), 14.22; FAB-MS (NBA/NaI): m/z 506 $[M + H]^+$; HRMS: calcd for $C_{21}H_{32}NO_{13}$ 506.1872, found 506.1874.

Typical Procedure for *N*-acylation of 5b. To compound **5b** (0.2 g, 0.32 mmol) dissolved in anhyd CH_2Cl_2 (5 mL) was added *i*-Pr_2NEt (0.25 mL) and 4-benzoylbenzoyl chloride (0.12 g, 0.49 mmol). After stirring for 12 h at rt, MeOH (5 mL) was added to the mixture while stirring it. The evaporation of volatile solvents afforded a pale red oily residue, which was purified with flash column chromatography (20 g silica gel) by eluting with 10% MeOH/CH_2Cl_2. Product **6d** (0.14 g, 60%) was obtained as an oil. $R_f = 0.66$ (10% MeOH/CH_2Cl_2). ^{1}H-NMR (250.13 MHz, CD_3OD): δ (ppm) 8.24−8.07 (m, 2H), 7.88−7.77 (m, 4H), 7.66−7.63 (br d, 1H), 7.57−7.51 (t, 2H, J = 7.7 Hz), 5.45−5.05 (m, 4H), 4.44−4.33 (m, 1H), 4.25−4.17 (q, 2H, J = 7.1 Hz), 4.14−4.0 (m, 2H), 2.58−2.50 (m, 1H), 2.10−1.9 (m, 16H), 1.30−1.23 (t, 3H, J = 7.1 Hz); FAB-MS (NBA/NaI): m/z 736 $[M + Na]^+$.

6a: Pale red oil (60%). ^{1}H-NMR (250.13 MHz, CD_3OD): δ (ppm) 5.40−5.36 (dd, 1H, J = 2.2, 6.6 Hz), 5.3−5.2 (m, 1H), 5.1−5.07 (m, 1H), 4.46−4.4 (dd, 1H, J = 2.5, 12.5 Hz), 4.28−4.17 (q, 2H, J = 7.0 Hz), 4.13−4.0 (m, 2H), 3.71 (s, 2H), 2.52−2.50 (dd, 1H), 2.15−1.84 (m, 16H), 1.34−1.23 (t, 3H, J = 7.0 Hz); FAB-MS (NBA/NaI): m/z 649 $[M + Na]^+$.

6b: Pale red oil (36%). ^{1}H-NMR (300.14 MHz, CD_3OD): δ (ppm) 6.24−6.05 (m, 2H), 5.68−5.65 (dd, 1H, J = 3.1, 8.7 Hz), 5.38−5.35 (dd, 1H, J = 2.4, 6.3 Hz), 5.27−5.22 (m, 2H), 5.10−5.05 (double t, 1H, J = 2.4, 6.3 Hz), 4.46−4.4 (dd, 1H, J = 2.4, 12.4 Hz), 4.27−4.18 (q, 2H, J = 7.2 Hz), 4.15−4.05 (m, 2H), 2.57−2.52 (dd, 1H, J = 5.1, 13.4 Hz), 2.10−1.90 (m, 16H), 1.38−1.33 (t, 3H, J = 7.0 Hz); FAB-MS (NBA/NaI): m/z 582 $[M + Na]^+$.

6c: Pale red oil (58%). ^{1}H-NMR (300.14 MHz, CD_3OD): δ (ppm) 8.03−8.0 (dd, 2H, J = 1.3, 6.5 Hz), 7.58−7.55 (dd, 1H, J = 1.3, 6.5 Hz), 7.48−7.42 (t, 2H, J = 6.5 Hz), 5.40−5.37 (dd, 1H, J = 2.4, 6.4 Hz), 5.36−5.23 (m, 2H), 5.12−5.07 (double t, 1H, J = 2.4, 6.4 Hz), 4.44−4.4 (dd, 1H, J = 2.5, 12.3 Hz), 4.25−4.20 (q, 2H, J = 7.2 Hz), 4.13−4.02 (m, 2H), 2.57−2.52 (dd, 1H, J = 4.9, 13.3 Hz), 2.14−1.90 (m, 16H), 1.27−1.23 (t, 3H, J = 7.2 Hz); FAB-MS (NBA/NaI): m/z 623 $[M + Na]^+$, 582 $[M - Et]^+$.

Acetylation of 4c. Compound **4c** (1.7 g, 3.13 mmol) and Ac_2O (20 mL) in 40 mL of pyridine was stirred for 48 h at rt. After concentration of the reddish reaction mixture, a pale yellow foam was obtained and purified with flash silica gel (100 g) chromatography eluting with 10% MeOH/CH_2Cl_2. The penta-*O*-acetylated product (**4c′**) was obtained in 72% yield (1.70 g) as light yellow oil. $R_f = 0.62$ (10% MeOH/CH_2Cl_2). ^{1}H-NMR (300.13 MHz, $CDCl_3$): δ (ppm) 7.35−7.27 (m, 5H), 5.48−5.46 (m, 1H), 5.36−5.29 (m, 1H), 5.25−5.22 (m, 1H), 5.08 (s, 2H), 5.05−5.02 (m, 1H), 4.49−4.43 (dd, 1H, J = 2.5, 12.4 Hz), 4.30−4.20 (q, 2H, J = 7.0 Hz), 4.19−4.12 (m, 2H), 3.18 (br s, 2H), 2.65−2.61 (dd, 1H, J = 5.1, 8.8 Hz), 2.57−2.50 (m, 2H), 2.13−2.01 (m, 15H), 1.58−1.49 (m, 4H), 1.29-1.15 (m, 5H); ^{13}C-NMR (100.55 MHz, $CDCl_3$): δ (ppm) 173.38, 170.75, 170.52, 170.29, 168.34, 128.42, 127.99, 77.39, 77.07, 76.75, 72.74, 71.45, 68.40, 67.86, 66.44, 62.36, 62.11, 48.89, 40.68, 36.17, 35.71, 29.35, 26.01, 24.65, 20.66, 13.71; FAB-MS (NBA/NaI): m/z 775 $[M + Na]^+$; HRMS: calcd for $C_{35}H_{48}N_2O_{16}$-Na 775.2898, found 775.2897.

Synthesis of 7. Compound **4c′** (1.5 g, 1.99 mmol) was solubilized in anhyd MeCN (20 mL), followed by dropwise addition of trimethylsilyl trifluoromethanesulfonate (0.65 mL, 4.21 mmol) at rt. The mixture was stirred at 50 °C for 2.5 h under a stream of N_2. After cooling to 0 °C, Na_2CO_3 (0.85 g) was added to the reaction mixture followed by stirring for 30 min at 0 °C. The solid material was filtered through a pad of Celite and washed with 20 mL of CH_2Cl_2. The filtrates were combined and concentrated *in vacuo* prior to flash silica gel (100 g) chromatography by eluting with 10% MeOH/CH_2Cl_2. The desired product with R_f of 0.54 (10% MeOH/CH_2Cl_2) was obtained as a pale yellow oil in 73% yield (0.92 g). ^{1}H-NMR (250.13 MHz, CD_3OD): δ (ppm) 7.34−7.20 (m, 5H), 6.36−6.35 (d, 1H, J = 4.1 Hz), 5.60−5.56 (dd, 1H, J = 2.5, 5.9 Hz), 5.39−5.36 (ddd, 1H, J = 2.5, 6.1 Hz), 4.98−4.93 (dd, 1H, J = 4.1, 8.6 Hz), 4.86 (s, 2H), 4.59−4.54 (dd, 1H, J = 2.5, 12.4 Hz), 4.29−4.20 (q, 2H, J = 7.2 Hz), 4.27−4.21 (m, 1H), 4.05−3.97 (t, 1H, J = 7.4 Hz), 3.55−3.50 (dd, 1H, J = 2.5, 9.8 Hz), 3.17−3.12 (t, 2H, J = 6.7 Hz), 2.36−2.30 (t, 1H, J = 7.4 Hz), 2.01−1.96 (m, 4H), 1.35−1.20 (m, 2H), 1.31−1.26 (t, 3H, J = 7.2 Hz); ^{13}C-NMR (100.55 MHz, $CDCl_3$): δ (ppm) 170.63, 170.50, 170.00, 169.60, 138.31, 128.64, 127.99, 127.77, 127.43, 107.18, 72.08, 70.79, 69.34, 66.44, 62.04, 61.79, 43.67, 42.83, 40.78, 39.97, 29.13, 27.89, 26.24, 25.33, 24.32, 23.15, 20.81, 14.10; FAB-MS (NBA/NaI): m/z 655 $[M + Na]^+$, 633 $[M + H]^+$; HRMS: calcd for $C_{31}H_{40}N_2O_{12}Na$ 655.2476, found 655.2479.

Synthesis of 8a. To oxazoline **7** (0.85 g, 1.34 mmol) dissolved in *t*-BuOH (15 mL) was added trimethylsilyl azide (0.71 mL, 5.35 mmol). After being stirred at 80 °C for 4 h under N_2 atmosphere, the mixture was concentrated *in vacuo* to yield a red oil, which was purified with flash silica gel (70 g) chromatography eluting with 10% MeOH/CH_2Cl_2. The product was obtained in 81% (0.74 g) as a pale red oil. $R_f = 0.66$ (10% MeOH/CH_2Cl_2). ^{1}H-NMR (300.14 MHz, CD_3OD): δ (ppm) 7.34−7.23 (m, 5H), 5.91−5.90 (d, 1H, J = 2.4 Hz), 5.42−5.40 (dd, 1H, J = 1.8, 4.6 Hz), 5.28−5.24 (m, 3H), 4.62−4.59 (dd, 1H, J = 3.5, 12.4 Hz), 4.42−4.41 (dd, 1H, J = 1.9, 10.6 Hz), 4.3−4.14 (m, 4H), 4.11−4.06 (t, 1H, J = 10.6 Hz), 3.20−3.18 (br m, 2H), 2.17−1.93 (m, 11H), 1.64−1.54 (quin, 2H, J = 7.3 Hz), 1.50−1.44 (quin, 2H, J = 7.3 Hz), 1.29−1.23 (m, 5H); ^{13}C-NMR (100.55 MHz, CD_3OD): δ (ppm) 173.33, 170.47, 170.22, 170.13, 161.05, 145.35, 138.24, 128.57, 128.40, 128.20, 127.86, 127.34, 107.62, 67.81, 66.38, 62.10, 61.65, 58.31, 47.62, 43.54, 35.30, 26.17, 23.06, 20.64, 13.91; FAB-MS (NBA/NaI): m/z 698 $[M + Na]^+$, 633 $[M - N_3]^+$.

Hydrolysis of 8a. To a solution of MeOH (5 mL) containing 0.7 g (1.04 mmol) of compound **8a** was added $LiOH\cdot H_2O$ (0.26 g, 6.20 mmol) dissolved in H_2O (10 mL). After stirring for 12 h at rt, the reaction mixture was acidified to pH ∼ 4 by adding cation exchange resin (Dowex 50W-X8; hydrogen form). The resins were filtered off, and the filtrate was evaporated prior to flash column chromatography with silica (10 g), eluting with 5% MeOH/CH_2Cl_2 to 3% HCO_2H/30% MeOH/CH_2Cl_2. From fractions with $R_f = 0.31$ (3% HCO_2H/20% MeOH/CH_2Cl_2), 0.34

g (62%) of **8b** was obtained as an oil (the oily residue was converted to powder after being dissolved in water and lyophilization). ^{1}H-NMR (300.14 MHz, CD_3OD): δ (ppm) 7.34−7.30 (m, 5H), 5.89−5.78 (d, 1H, J = 2.3 Hz), 5.05 (s, 2H), 4.32−4.24 (m, 2H), 4.18−4.14 (t, 1H, J = 9.5 Hz), 3.91−3.89 (m, 2H), 3.13−3.08 (t, 2H, J = 7.1 Hz), 2.30−2.25 (t, 2H, J = 7.1 Hz), 1.66−1.64 (m, 2H), 1.51−1.49 (m, 2H), 1.38−1.35 (m, 2H); ^{13}C-NMR (100.55 MHz, CD_3OD): δ (ppm) 177.14, 168.57, 158.85, 150.04, 138.38, 129.41, 128.89, 128.69, 77.20, 67.27, 63.74, 59.44, 41.59, 40.13, 37.06, 30.53, 30.05, 27.33, 26.66, 26.47; FAB-MS (glycerol): m/z 560 $[M + K]^+$, 544 $[M + Na]^+$; HRMS: calcd for $C_{23}H_{31}N_5O_9Na$ 544.2018, found 544.2019.

Deprotection of *N*-Cbz Group of 8b. Compound **8b** (0.2 g, 0.384 mmol) was dissolved in CF_3CO_2H/CH_2Cl_2 (1/1; 2 mL) containing anisole (62 mg, 0.576 mmol), followed by addition of CF_3SO_3H (0.34 mL, 3.84 mmol) at 0 °C. After stirring for 30 min at 0 °C, 3 mL of water was added to the reaction mixture. This aqueous mixture was washed with CH_2Cl_2 (3 × 5 mL), neutralized with Na_2CO_3, and evaporated to a solid material. The solid residue was extracted with MeOH (10 mL). The extract was concentrated before being applied to a flash silica gel (10 g) column, which was eluted with 5% MeOH/CH_2Cl_2 to 3% HCO_2H/50% MeOH/CH_2Cl_2. The product **8c** was obtained as a pale red oil (50 mg, 33%). This oily material, after being dissolved in water and lyophilization, was converted to a pale brown solid (hygroscopic). R_f = 0.31 (5% HCO_2H/30% MeOH/CH_2Cl_2). ^{1}H-NMR (500.14 MHz, D_2O): δ (ppm) 5.61 (d, 1H, J = 1.7 Hz), 4.23−4.18 (t, 2H, J = 10.7 Hz, H_4, H_5), 4.11−4.08 (t, 1H, J = 10.0 Hz, H_6), 3.84−3.81 (m, 1H, H_8), 3.79−3.75 (dd, 1H, J = 11.8 Hz, H_9), 3.56−3.52 (pseudo t, 2H, H_7, $H_{9'}$), 3.07−3.04 (t, 2H, J = 5.8 Hz), 2.24−2.21 (t, 1.3 H, J = 6.8 Hz), 2.13−2.11 (t, 0.7 H, J = 6.8 Hz), 1.56−1.53 (m, 2H), 1.45−1.40 (m, 2H), 1.26−1.20 (m, 2H); ^{13}C-NMR (100.61 MHz, D_2O/CD_3OD): δ (ppm) 103.93, 76.22, 69.15, 64.13, 60.26, 40.20, 36.77, 29.03, 26.63, 25.90, 22.73; FAB-MS (negative ion mode): m/z 386 $[M - 1]^-$.

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Supporting Information Available: Copies of ^{1}H NMR spectra of compounds **2a**−**e**, **3a**−**e**, **4a**−**d**, **5a**, **6a**,**c**,**d**, **7**, and **8a**−**c** (22 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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