Pergamon

Tetrahedron Letters, Vol. 38, No. 18, pp. 3127-3130, 1997

0040-4039/97 $17.00 + 0.00

PII: S0040-4039(97)00569-8

Synthesis of Peptidosialosides And Peptidosaccharides

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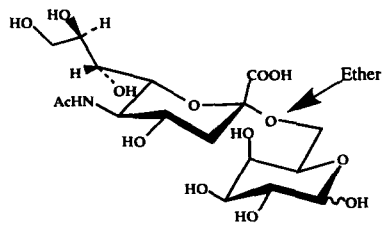
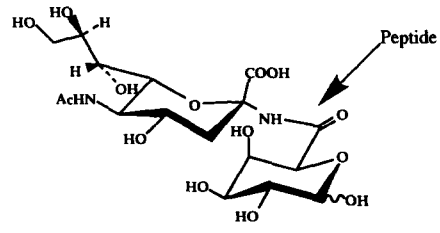
Abstract: Synthesis of a sialoside and neutral sugars connected to a galactose uronic acid by a peptide bond is described. © 1997 Elsevier Science Ltd.

Oligosaccharides serve as receptor determinants in numerous biological reactions and infectious processes. Inspite of our understanding of carbohydrate structure and function, the discovery of carbohydrate based drugs has been difficult due to the lack of economical syntheses of oligosaccharides and their analogs by chemical, enzymatic, and chemoenzymatic methods. This limitation stems from the difficulty in establishing the glycosidic ether linkage stereoselectively. In addition, the glycosidic linkages so constructed are the break down targets for glycohydrolases present *in vivo*. Recently, attempts have been made to connect sugars via a peptide linkage.[1-3] This seems to be an attractive strategy, since numerous methods are available for the quantitative chemical construction of peptide bonds and further more, such peptide linked saccharides may be stable to glycosidases. To this end, we report here a methodology for the preparation of a peptide linked sialoside (**2**), which is a mimic of an αDNeuAc(2-6)βDGal (**1**) unit that is found in numerous glycoproteins and glycolipids. Since **1** is a receptor ligand for influenza virus hemagglutinin and the neuraminidase, the mimic **2** is a potential influenza virus inhibitor.[4] Besides the sialoside **2**, we have prepared peptidodisaccharides containing glucose, N-acetylglucosamine, fucose, and mannose to demonstrate the generality of this method. This is the first report, where the nitrogen of the peptide bond in the disaccharide is connected to the anomeric carbon, instead of the aglycon and this is expected to be sterically better compatible with that of a glycosidic oxygen.



αDNeuAc(2-6)ßDGal (1)

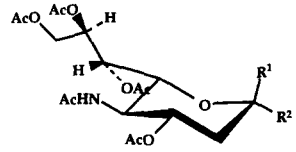


αDNeuAc(N-2-6-CO)ßDGal (2)

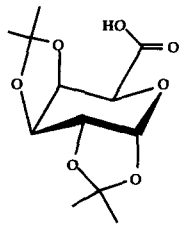
The synthesis of peptidosaccharides involved the coupling of a glycosylamine with an activated glycosyluronic acid. The glycosyl amines were prepared readily by the reduction of glycosyl azides, the

preparation of which has been reported earlier by us.[5] The activated uronic acids were prepared by reacting the uronic acid with the activating agent 1,1-carbonylbis(3-methylimidazolium triflate) (CBMIT), a reagent developed by Saha et al. for use in the peptide synthesis.[6] This methodology afforded high quality peptidosaccharides.

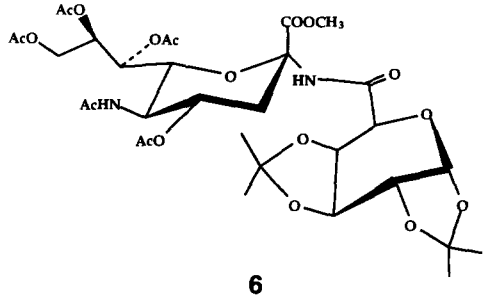
The α-azido sialic acid **4** was prepared from the β-chloride[4] **3** by treatment with sodium azide. The aglycone 1,2,3,4-di-O-isopropylidene-α-D-galactopyranose)uronic acid **5** was prepared in one step by ruthenium catalyzed oxidation[7] of the commercially available aglycone 1,2,3,4-di-O-isopropylidene-α-D-galactopyranose. Carboxylic acid **5** was activated with CBMIT instantly (5 min, as evidenced from the n.m.r. spectrum). The hydrogenation of the glycosyl azide **4** with palladium on carbon and subsequent addition to the activated carboxylate of **5** provided the peptidosialoside **6** as a single anomer. The ^{1}H- and ^{13}C-n.m.r were consistent with that expected for an α -sialosidic linkage.[8,9] The peptide linkage was stable to acidic and basic conditions, as evidenced from the preparation of the unprotected disaccharide **2** via the removal of the isopropylidene group of **6** with 50% aqueous trifloroacetic acid, the acetate groups with sodium methoxide in methanol and the hydrolysis of the methyl ester with sodium Chelex Resin.[4]



3, R^1 = Cl, R^2 = $COOCH_3$
4, R^1 = $COOCH_3$, R^2 = N_3



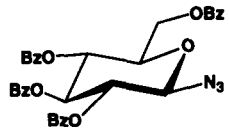
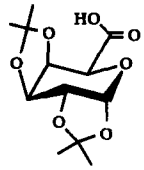
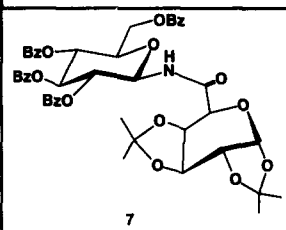
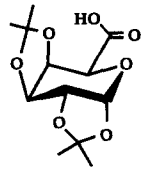
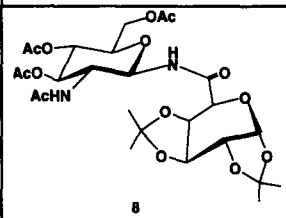
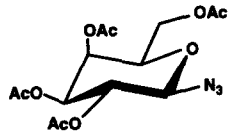
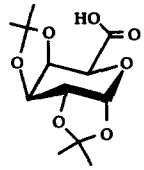
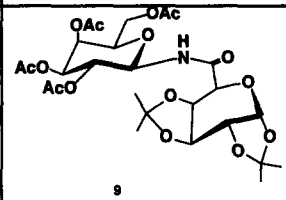
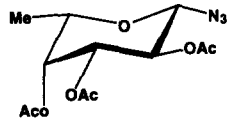
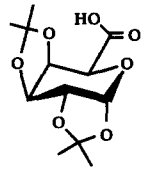
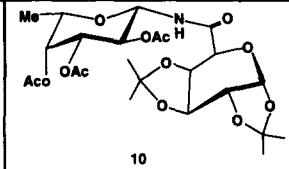
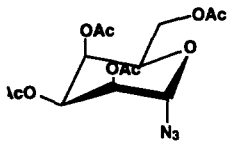
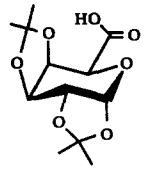
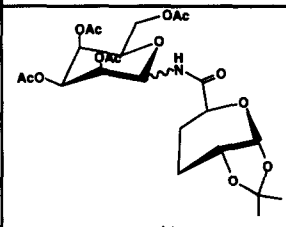
5



6

Compared to the sialoside **6**, the coupling of amines derived from the azides of glucose, galactose, mannose, fucose and including that of 2-acetamido-2-deoxy-glucose (Table) with that of activated **5** was even faster (2 h). In each case, the crude product obtained was sufficiently pure, as evidenced from their n.m.r spectra. Also, the protecting groups were removed from these disaccharides under standard conditions without destroying the peptide linkage. It is to be noted that the equatorial placement of the nitrogen of the peptide bond at the anomeric carbon is synthetically advantageous, as the glycosyl amines naturally tend to prefer equatorial orientation. As a result, there is no loss of stereochemical integrity at the glycosidic center during peptide formation. This natural preference also coincides with the α-configuration found to date for all the natural sialosides. The limitation however is that the peptidosaccharide mimics of α -glycosides with axially

Table. The list of peptidodisaccharides synthesized

Entry	Glycosyl Azide	Uronic acid	Product	% Yield
1		 5	 7	78[a]
2		 5	 8	95[b]
3		 5	 9	78[b]
4		 5	 10	71[a]
5		 5	 11	89[b] (α:β = 3:2)

a: Purified product;. b: Crude product

oriented aglycon will be difficult to construct with stereochemical integrity. This was evidenced by the formation of anomeric mixtures of peptidosaccharides **11**, when the α -mannosyl azide was hydrogenated and coupled to the uronic acid **5** (Entry 5). The binding properties of the sialoside mimic **2** to sialic acid binding proteins, as well as of those mentioned in the Table to plant lectins will be carried out in due course.

To conclude, the methodology described here should lead to a new generation of oligosaccharide mimics. The ease of synthesis should make this method suitable for a solution based combinatorial chemical synthesis of oligosaccharides mimics. More importantly, as in the case of peptides, automation of the peptidooligosaccharide synthesis can be achieved by pre-synthesizing the required glycosyl azide or glycosyl amine and the aglycone carboxylic acid.

REFERENCES AND NOTES

1. Nicalaou, K. C.; Florke, H.; Egan, M. G.; Bath, T.; Estevez, V. A. *Tetrahedron Lett.* **1995,** *36*, 1775.
2. Suhara, Y.; Hildreth, J. E. K.; Ichikawa, Y. *Tetrahedron Lett* **1996,** *37*, 1575-1578.
3. Muller, C.; Kitas, E.; Wessel, H. P. *J Chem Soc Chem Commun* **1995**, 2425-2426.
4. Sabesan, S.; Neira, S.; Davidson, F.; Duus, J. Ø.; Bock, K. *J. Am. Chem. Soc.* **1994,** *116*, 1616.
5. Sabesan, S.; Neira, S. *Carbohydrate Research* **1992,** *223*, 169-185.
6. Saha, A. K.; Schultz, P.; Rapoport, H. *J. Am. Chem. Soc.* **1989,** *111*, 4856 - 4859.
7. Mio, S.; Kumagawa, Y.; Sugai, S. *Tetrahedron* **1991,** *47*, 2144.
8. Rothermel, J.; Weber, B.; Faillard, H. *Liebigs Ann. Chem.* **1992,** 799-802.
9. Synthesis of **6**: Azide **4** (726 mg) in acetonitrile (60 ml) was hydrogenated with 10% palladium on carbon (96 mg, 45 min, Step 1). Carboxylic acid **5** (410 mg) in acetonitrile (10 ml) was activated with 1,1'-Carbonylbis(3-methylimidazolium triflate) (CBMIT, 780 mg, 5 min) and added to the filtrate from step1 and stirred under dry nitrogen atmosphere for 16 h. Additional portion of carboxylic acid (210 mg) activated with CBMIT (390 mg) in acetonitrile (5 ml) was added and the reaction was continued for another 72 h. The reaction mixture was concentrated under reduced pressure, the residue dissolved in methylene chloride, washed with water, ice-cold 0.5 M HCl, sat. sodium bicarbonate solution, dried and concentrated to dryness. The product was purified by chromatography on a column of silica gel using ethylacetate as eluant. Weight of the product **6** was 583 mg. ^{1}H-NMR ($CDCl_3$) ∂: 5.63 (d, H-1), 5.33 (dd, H-7'), 5.26 (d, NH), 5.22 (m, H-8'), 5.11 (m, H-4'), 4.65 (dd, H-3), 4.58 (dd, H-4), 4.36 (dd, H-9'), 4.28 (dd, H-6'), 4.25 (d, H-5), 3.79 (s, $COOCH_3$), 2.92 (H-3'eq), 2.12, 2.08, 2.03, 1.89 (5 x CH_3CO), 1.98 (dd, H-3'ax), 1.53, 1.45, 1.35, 1.32 (isopropylidene methyls). ^{13}C-NMR ($CDCl_3$) ∂: 170.9, 170.5, 170.3, 170.0, 168.4, 109.6, 109.4, 96.2, 83.3, 73.3, 71.7, 70.7, 70.6, 70.4, 70.3, 69.3, 68.9, 67.7, 61.7, 52.9, 49.5, 37.5, 26.0, 25.8, 24.8, 24.3, 23.19, 21.0-20.8.

(*Received in USA* 31 *January* 1997; *accepted* 19 *March* 1997)