Tetrahedron Letters,Vol.27,No.47,pp 5739-5742,1986 0040-4039/86 $3.00 + .00
Printed in Great Britain Pergamon Journals Ltd.

TOTAL SYNTHESIS OF A UNDECASACCHARIDE: A TYPICAL CARBOHYDRATE SEQUENCE FOR THE COMPLEX TYPE OF GLYCAN CHAINS OF A GLYCOPROTEIN[1)]

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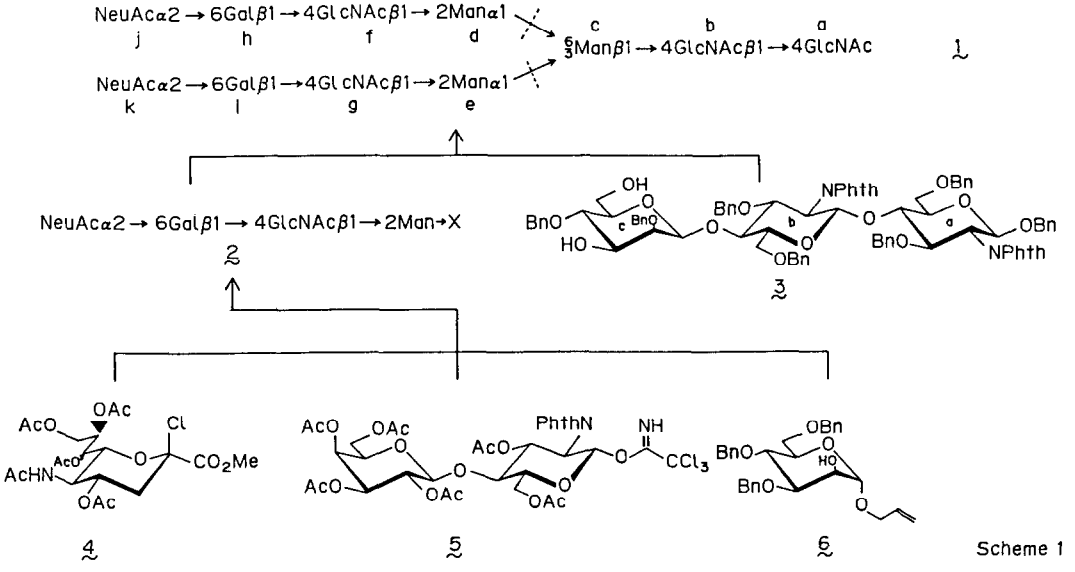
Abstract: A first total synthesis of undecasaccharide 1 of complex type of glycans of a glycoprotein was achieved in a stereo- and regiocontrolled way.

The glycan part of glycoproteins is now known to play significant roles for manifesting biological functions of the glycoproteins which control cellular regulation and recognition[2]. The glycan 1 is classified as a typical complex type of glycans[3] which is linked covalently to an Asn residue of proteins. As part of our project on the synthesis of glycans of glycoproteins, we describe here a total synthesis of the glycan 1. In close connection with our project, an independent approach to the synthesis of N-linked glycans of glycoproteins has actively been pursued by Paulsen and his co-workers[4].

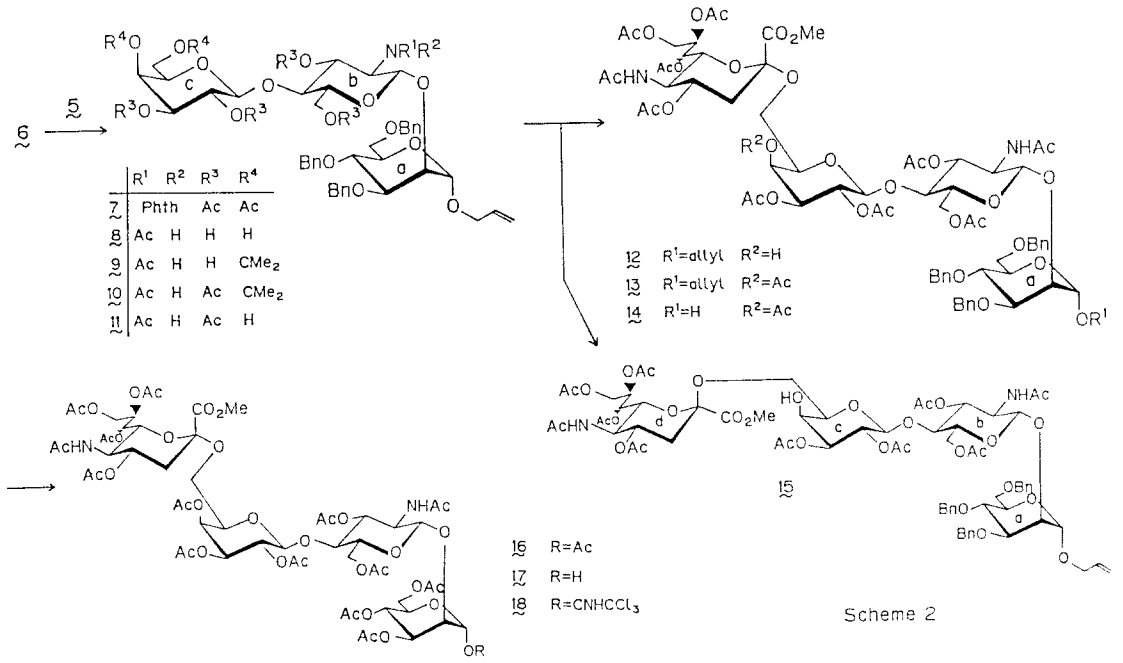
Retrosynthetic analysis of undecasaccharide 1 led us to design a glycotetraosyl donor 2 which correspond to two symmetric branches in 1, and a glycosyl acceptor 3 which was prepared previously[5]. The glycotetraosyl donor 2 was planned to be synthesized from two glycosyl donors 4[6] and 5[7], and a glycosyl acceptor 6[8].



Scheme 1

Glycosylation of compound 6 with the imidate 5 in the presence of $BF_3 \cdot Et_2O$[9]-molecular sieves AW-300 in dichloroethane afforded a 73% yield of protected trisaccharide 7, $[\alpha]_D$ -2.7° (c 1.3)[10], Rf 0.38 in 2:1 toluene-EtOAc, δ_H[11] 5.507 (d, 1 H, J 8.6 Hz, H-1b). Conversion of compound 7 into N-acetyl derivative 8, Rf 0.41 in 3:1 $CHCl_3$-MeOH, δ_H 1.93 (s, 3 H, NAc), was

performed in 4 steps in 79% overall yield: (1) NaOMe-MeOH, (2) 1:5 $nBuNH_2$-MeOH,[12] reflux, (3) Ac_2O-pyridine, and (4) NaOMe-MeOH. Isopropylidenation of compound **8** with $Me_2C(OMe)_2$-TsOH-DMF at 0° followed by acetylation afforded a 67% yield of the desired kinetic product **10**, $[\alpha]_D$ +20.8° (c 0.9), Rf 0.35 in 1:3 toluene-EtOAc, δ_H 1.42 and 1.36 (2 s, 6 H, CMe_2), δ_C 98.94 ($\underline{C}Me_2$), as well as a 14% yield of 3,4-O-isopropylidene isomer, Rf 0.44 in 1:3 toluene-EtOAc. Solvolysis of compound **10** in 1:1 AcOH-MeOH at 80° gave an 89% yield of diol **11**, $[\alpha]_D$ +11.3° (c 0.6), Rf 0.49 in 1:1 CH_2Cl_2-acetone, which was glycosylated with the chloride **4** in the presence of $HgBr_2$-$Hg(CN)_2$-molecular sieves 4A in $Cl(CH_2)_2Cl$. α-Linked product **12**, $[\alpha]_D$ -2.6° (c 0.9), Rf 0.44 in 10:1 $CHCl_3$-MeOH, δ_H 2.591 (dd, 1 H, J 4.7 and 12.9 Hz, H-3deq) was isolated in 48% yield along with β-isomer **15** (33%), $[\alpha]_D$ +2.1° (c 1.0), Rf 0.47 in 10:1 $CHCl_3$-MeOH, δ_H 2.464 (dd, 1 H, J 5.1 and 13.2 Hz, H-3deq).

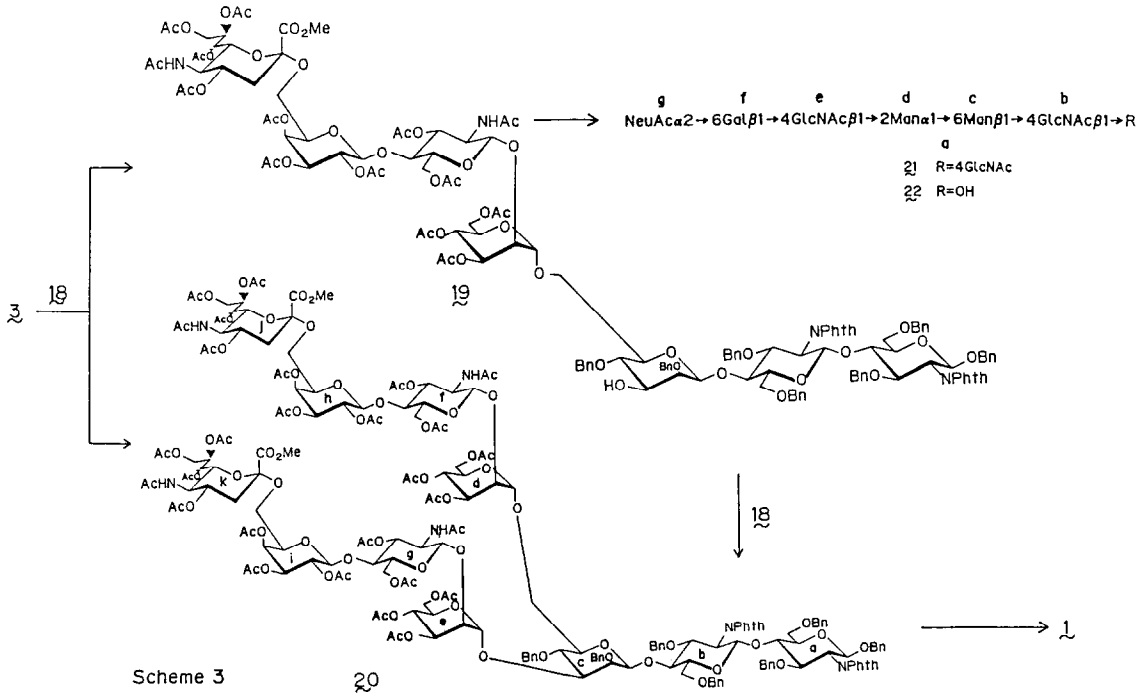


Scheme 2

Tetrasaccharide derivative **12** was transformed into a glycosyl donor **18**, a synthetic equivalent with the glycotetraosyl donor **2** depicted in Scheme 1. Acetylation of compound **12** gave a 95% yield of nonaacetate **13**, $[\alpha]_D$ -11.4° (c 0.9), which was deallylated with $PdCl_2$-NaOAc-aq.AcOH[13] to give a 55% yield of hemiacetal **14**, $[\alpha]_D$ -19.8° (c 0.6), Rf 0.55 in 1:1 CH_2Cl_2-acetone. Hydrogenolysis of compound **14** in the presence of 10% Pd-C in 10:1 MeOH-AcOH, followed by acetylation afforded an 83% yield of peracetate **16**, $[\alpha]_D$ -25.5° (c 0.9), Rf 0.52 in 9:1 $CHCl_3$-MeOH, δ_H 6.019 (d, 1 H, J 1.5 Hz, H-1a), 4.866 (ddd, 1 H, J 4.6, 9.1, and 10.6 Hz, H-4d) and 2.537 (dd, 1 H, J 4.6 and 12.9 Hz, H-3deq). Chemoselective deacetylation of compound **16** with NH_2NH_2•AcOH in DMF[14] and subsequent treatment of the resulted hemiacetal **17** with Cl_3CCN and DBU in $Cl(CH_2)_2Cl$ afforded an 85% yield of the designed glycosyl donor **18**, $[\alpha]_D$ -19.4° (c 1.6), Rf 0.54 in 9:1 $CHCl_3$-MeOH, δ_H 8.694 (s, 1 H, =NH), and 6.203 (d, 1 H, J 1.7 Hz, H-1a), δ_C 95.1 ($^1J_{CH}$ 175 Hz, C-1a).

Having the key glycosyl donor **18** prepared, we now describe crucial glycosylation of the key acceptor **3** with the donor **18**. We expected that glycosylation at a primary hydroxyl group

(C-6c OH) of compound 3 may proceed smoothly but the reaction at C-3c OH might be sluggish. Therefore, selective monoglycosylation of the acceptor 3 was first examined. A mixture of compound 3 and 0.77 equivalent of the glycosyl donor 18 was stirred in the presence of $BF_3 \cdot Et_2O$-molecular sieves AW-300 at -10° and flash chromatography of the products over silica gel in 25:1 $CHCl_3$-MeOH afforded a 48% yield (based on the donor 18) of the monoglycosylated product 19, $[\alpha]_D$ -12.2° (c 1.0), Rf 0.62 in 9:1 $CHCl_3$-MeOH. 400 MHz ^{1}H-nmr revealed signals of 43 protons for aromatic protons and 42 protons for 14 acetyl methyl protons as well as a singlet for methyl ester at δ 3.794, supporting that the product 19 was a monoglycosylated one. The structure of compound 19 was further confirmed by conversion into free heptasaccharide 21, $[\alpha]_D$ +7.7° (c 0.1, H_2O), Rf 0.18 in 2:1:1 nBuOH-EtOH-H_2O in 4 steps in 44% overall yield: (1) LiI-pyridine, 120°, (2) $NH_2NH_2 \cdot H_2O$-EtOH, reflux, (3) Ac_2O-MeOH, and (4) 10% Pd-C in 7:3 MeOH-H_2O. 400 MHz ^{1}H-Nmr of compound 21 in D_2O showed following signals: δ (60°) 5.175 (d, 0.5 H, J 2.4 Hz, H-1aα), 4.906 (s, 1 H, H-1d), 4.726 (s, 1 H, H-1c), 4.685 (d, 0.5 H, J 8.0 Hz, H-1aβ), 4.595 (m, 2 H, H-1b and H-1e), 2.663 (dd, 1 H, J 4.4 and 12.2 Hz, H-3geq), 2.057, 2.040, 2.033, 2.019 (4 s, 12 H, 4NAc), and 1.667 (t, 1 H, J 12.5 Hz, H-3gax), in good agreement with the data for related natural hexasaccharide 22[15].



Scheme 3

Since regio- and stereochemistry of compound 19 was established, further elongation of a glycan chain at C-3c OH of the heptasaccharide 19 was examined and, under the same condition by using 1.3 equivalents of the glycosyl donor 18, was obtained, after purification by high pressure gel permeation chromatography (GPL 220 column, HITACHI) in $CHCl_3$, a 56% yield of the desired protected undecasaccharide 20, $[\alpha]_D$ -7.8° (c 0.4), Rf 0.12 in 1:1 CCl_4-acetone, δ_H 3.803 and 3.789 (2 s, 6 H, OMe). Compound 20 was deblocked in 4 steps as in the case of 19 to give free undecasaccharide 1, $[\alpha]_D$ -4.6° (c 0.05, H_2O), Rf 0.14 in 2:1:1 nBuOH-EtOH-H_2O in 15% overall yield. 400 MHz ^{1}H-Nmr of compound 1 in D_2O contained signals at δ (60°) 5.185 (d, 0.5

H, J 2.2 Hz, H-1aα), 5.128 (s, 1 H, H-1e), 4.925 (s, 1 H, H-1d), 4.753 (s, 1 H, H-1c), 4.695 (d, 0.5 H, J 7.4 Hz, H-1aβ), 4.602 (d, 3 H, J 7.5 Hz, H-1b, H-1h and H-1g), 4.226 (H-2c), 4.173 (H-2e), 4.090 (H-2d), 2.674 (dd, 2 H, J 4.2 and 12.2 Hz, H-3jeq. and H-3keq), and 1.679 (t, 2 H, J 11.7 Hz, H-3jax and H-3kax). These observed data for synthetic **1** was found to be in good agreement with related oligosaccharides isolated from glycoproteins[15].

In conclusion, a regio- and stereocontrolled total synthesis of undecasaccharide **1**, a typical carbohydrate sequence for complex type of glycans of glycoproteins, was achieved for the first time. Comparison of ^{1}H-nmr of synthetic **1** and **21** with those of naturally occurring glycans isolated from glycoproteins provided unambiguous synthetic evidences for the proposed structures of this class of glycans.

Aknowledgment. We are indebted to Mr. Shitori of MECT Co. for a generous supply of N-acetylneuraminic acid. We thank Dr. J. Uzawa and Mrs. T. Chijimatsu for recording and measuring the NMR spectra and Dr. H. Honma and his staff for the elemental analyses. We also thank Ms. A. Takahashi and Ms. K. Moriwaki for their technical assistance.

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(Received in Japan 7 August 1986)