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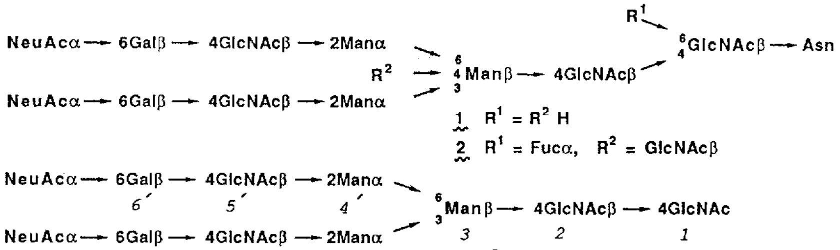
STEREOSELECTIVE SYNTHESIS OF A CORE GLYCOHEPTAOSE OF BISECTED BIANTENARRY COMPLEX TYPE GLYCAN OF GLYCOPROTEINS[1]

Fumito Yamazaki, Tomoo Nukada, Yukishige Ito, Susumu Sato, and Tomoya Ogawa*
RIKEN (The Institute of Physical and Chemical Research), Wako-shi, Saitama, 351-01 Japan

Abstract: A stereocontrolled synthesis of a core glycoheptaose of "bisected" complex type glycans of a glycoprotein was achieved by use of stereoselective glycosylation.

Complex type N-linked glycans[2] of glycoproteins may be classified into two groups, complex type such as **1** and "bisected" complex type such as **2**. The "bisecting" GlcNAc residue at 4-O of Manβ residue has been found in the glycan of glycoproteins isolated from tissues[3] such as hematopoietic cells, kidney, oviduct and malignant tissues. The biological role of bisecting GlcNAc has been discussed in terms of glycan conformaiton[4] as well as biosynthetic regulation[5].



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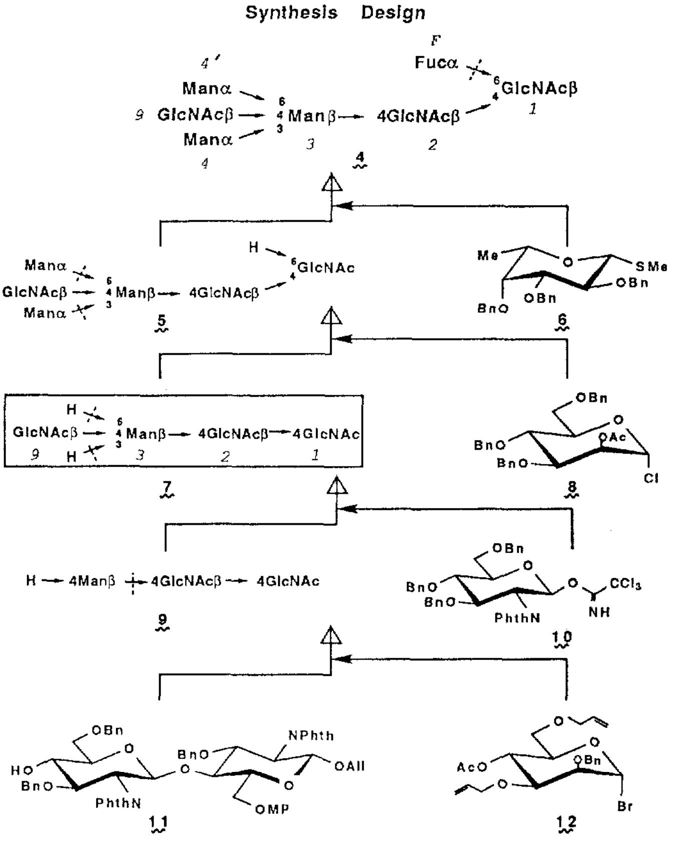
In 1986, we reported a stereocontrolled synthesis[6] of undecasaccharide **3** that correspond to the glycan part of **1**. As part of our project on synthetic studies of complex type glycans of glycoproteins, we describe here a synthetic approach to a core glycoheptaose **4** present in various "bisected" complex type glycans. In close connection with our results, it is to be noted that different approaches to the synthesis of "bisected" glycooligoses were recently reported[7,10].

The target molecule **4** was disconnected stepwise into four monosaccharide glycosyl donors **6**, **8**, **10**, **12** and a disaccharide glycosyl acceptor **11** as shown in Scheme 1 based on retrosynthetic analysis. Since glycosyl donors **6**[8,12], **8**[9] and **12**[10] have been repoted, efficient preparative routes to a glcosyl donor **10** and a glycosyl acceptor **11** were first examined (Scheme 2). Readily available diol **13**[11] was converted into trichloroacetimidate **10**[12] in 3 steps in 55% overall yield (1 Ag_2O-KI-BnBr in DMF, 2 $PdCl_2$-AcONa-aq.AcOH[13], 3 Cl_3CCN-DBU in $(CH_2Cl)_2$[14]). Two glycosyl synthons **14** and **15** required for the preparation of the glycosyl acceptor **11** were obtainable from diol **13** as follows. Treatment of **13** with MeOPhOH-Ph_3P-DEAD[15] in CH_2Cl_2 afforded an 84% yield of **14**[12]. Conversion of **13** into **15**[12] was achieved in 4 steps in 50% overall yield (1 $(Bu^n{}_3Sn)_2O$[16], then BnBr-$Bu^n{}_4NBr$[17], 2 Ac_2O-pyridine, 3 $PdCl_2$-AcONa-aq.AcOH, 4 CCl_3CN-DBU in $(CH_2Cl)_2$). Coupling between **14** and **15** in the presence of $BF_3{\cdot}Et_2O$-MS-AW300 in $(CH_2Cl)_2$ at -23° according to Schmidt[18] and subsequent deacetylaiton by NaOMe-MeOH afforded the chitobiosyl glycosyl acceptor **11**[12] in 73% overall yield.

Glycosylation of **11** with properly protected mannosyl donor **12** in the presence of Ag silicate-MS4A in $(CH_2Cl)_2$ according to Paulsen[19] gave β-glycoside **16**[12] and α-isomer **18**[12] in 48 and 19% yield, respectively. The formation of the β-glycoside **16** as a major product in a ratio of 2.5:1 may be explained as a substituent effect[20] of 4-O-acetyl in the donor **12**, since an analogous glycosyl donor, 3,6-di-O-allyl-2,4-di-O-benzyl-α-

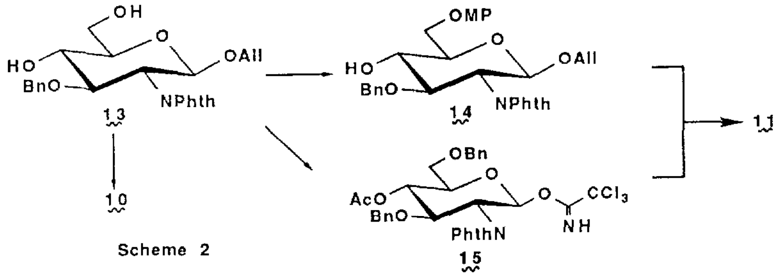


Scheme 1 (All = allyl, MP = MeOPh)

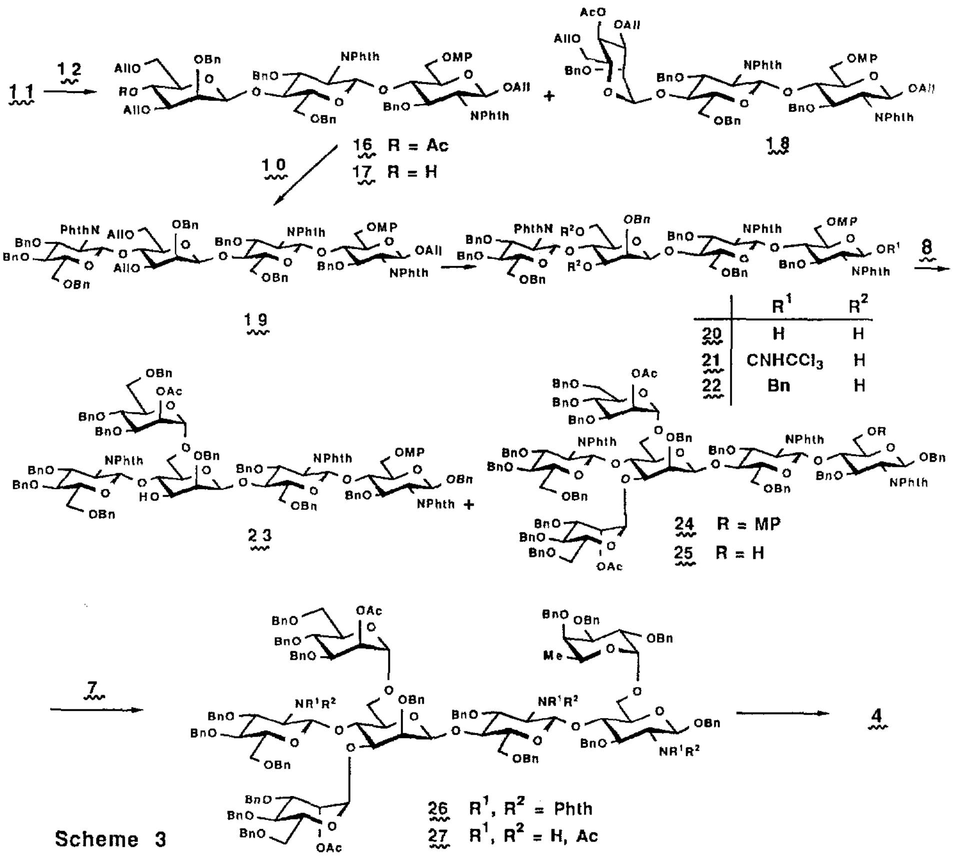
D-mannopyranosyl bromide upon reaction with a similar glycosyl acceptor gave[21] β- and α-glycoside in a ratio of 1:1. Deacetylation of **16** by LiOH-H_2O_2 in THF gave a 98% yield of **17**[12] which upon glycosylation (BF_3•Et_2O-MS-AW300) with the imidate **10** gave a 94% yield of **19**[12]. Conversion of **19** into tetrasaccharide glycosyl acceptor **22**[12], that corresponds to **7** in Scheme 1, was done in 3 steps via **20**[12] and **21**[12] in 64% overall yield (1 $(Ph_3P)_3RhCl$-DABCO, then $HgCl_2$-HgO[22], 2 Cl_3CCN-DBU in CH_2Cl_2 at -55°, 3 BF_3•Et_2O-MS-AW300-BnOH in $(CH_2Cl)_2$). Silver triflate promoted glycosylation of **22** with large excess of mannosyl donor **8**[9] which is suitably protected in order to elongate glycan chain further at O-2 for the synthesis of such extended glycans as **2**, gave mono- and diglycosylated product **23**[12] and **24**[12] in 34 and 37% yield, respectively. Pentasaccharide **23** was glycosylated again with **8** to give a 36% yield of **24**.

Selective deprotection of **24** was achieved[23] by $(NH_4)_2Ce(NO_3)_6$ in 8:1 CH_3CN-H_2O to give a 74% yield of **25**[12] corresponding to **5** in Scheme 1. Highly stereoselective glycosylation of **25** with thioglycoside **6** in the presence of $CuBr_2$-$Bu^n{}_4NBr$[25] in $(CH_2Cl)_2$-DMF afforded the desired glycoheptaoside **26**[12] in 77% yield. Deprotection of **26** via **27**[12] into **4**[12] was achieved in 4 steps in 90% overall yield (1 NH_2NH_2•H_2O-EtOH 80°, 2 Ac_2O-pyridine-DMAP, 3 NaOMe-MeOH, 4 10% Pd/C-H_2 in MeOH). ^1H N.m.r. data of **4** was in good agreement with those[25] of related glycans isolated from natural sources.

In conclusion, a versatile and stereocontrolled synthetic route to a core glycoheptaose **4** of "bisected" complex type glycan **2** of glycoproteins has been established by employing a key glycotetraosyl intermediate **22**.



Scheme 2



Scheme 3

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12) Physical data for key compounds are described below. Values of $[\alpha]_D$ and $\delta_{H,C}$ were measured for $CHCl_3$ and $CDCl_3$ solutions, respectively, at 25°, unless noted otherwise.
4: δ_H (D_2O, 50°) 5.207 (d, 1.5 Hz, H-1^{4}), 5.171 and 4.682 (2d, 2.9 and 8.2 Hz, H-$1^{1}\alpha\beta$), 4.930 (d, 1.5 Hz, H-$1^{4'}$), 4.882 and 4.873 (2d, 3.1 and 3.9 Hz, H-1^{F}), 4.733 (s, H-1^{3}), 4.651 (d, 7.0 Hz, H-1^{2}), 4.167 (d, 2.7 Hz, H-2^{3}), 4.133 (dd, 1.8 and 3.3 Hz, H-2^{4}), 4.078 (q, 6.8 Hz, H-5^{F}); δ_H (D_2O) 5.216 (s, H-1^{4}), 5.165 (d, 2.7 Hz, H-$1^{1}\alpha$), 4.930 (d, 1.3 Hz, H-$1^{4'}$), 4.882 and 4.873 (2d, 3.0 and 3.5 Hz, H-1^{F}), 4.502 (d, 8.1 Hz, H-1^{9}), 4.173 (s, H-2^{3}), 4.134 (s, H-2^{4}), 4.088 (q, 6.7 Hz, H-5^{F}), 2.078, 2.042, 2.023 (3s, NHAc x 3), 1.205 and 1.194 (2d, 6.1, 6.8 Hz, H-6^{F}). **6**: $[\alpha]_D$ -0.2° (c 1.5); δ_H 4.300 (d, 9.7 Hz, H-1), 2.206 (s, SCH_3), 1.211 (d, 6.4 Hz, H-6). **10**: $[\alpha]_D$ +75.3° (c 1.2); δ_H 8.543 (s, C=NH), 6.418 (d, 8.5 Hz, H-1). **11**: $[\alpha]_D$ +35.0° (c 1.1); δ_H 5.272 (d, 7.9 Hz, H-1^{2}), 5.038 (d, 8.2 Hz, H-1^{1}), 3.791 (s, OCH_3). **14**: $[\alpha]_D$ +21.8°; δ_H 5.221 (d, 8.2 Hz, H-1), 3.772 (s, OCH_3). **15**: δ_H 8.580 (s, C=NH), 6.439 (d, 8.8 Hz, H-1), 1.943 (s, Ac). **16**: $[\alpha]_D$ +20.8° (c 1.1); δ_H 5.261 (d, 8.2 Hz, H-1^{2}), 5.043 (d, 7.6 Hz, H-1^{1}), 4.536 (s, H-1^{3}); δ_C 101.1 ($^{1}J_{CH}$ 158 Hz, C-1^{3}), 97.4 and 97.0 (C-$1^{1,2}$). **17**: $[\alpha]_D$ +14.9° (c 0.9); δ_H 5.260 (d, 8.2 Hz, H-1^{2}), 5.038 (d, 7.6 Hz, H-1^{1}), 4.522 (s, H-1^{3}), 3.234 (td, 5.0, 9.5 Hz, H-5^{3}), 3.054 (dd, 2.7, 9.5 Hz, H-3^{3}). **18**: $[\alpha]_D$ +45.8° (c 1.1); δ_H 5.216 (d, 7.9 Hz, H-1^{2}), 5.020 (d, 7.9 Hz, H-1^{1}); δ_C 100.2 ($^{1}J_{CH}$ 174 Hz, C-1^{3}), 97.1 (C-$1^{1,2}$). **19**: $[\alpha]_D$ +17.4° (c 0.9); δ_H 5.349 (d, 8.6 Hz, H-1^{9}), 5.184 (d, 8.2 Hz, H-1^{2}), 5.009 (d, 7.9 Hz, H-1^{1}), 4.346 (s, H-1^{3}). **20**: $[\alpha]_D$ +49.8° (c 0.8); δ_C 100.4 (C-1^{3}), 98.6, 97.5 (C-$1^{2,9}$), 92.5 (C-1^{1}). **21**: δ_H 8.406 (s, C=NH), 6.253 (d, 7.5 Hz, H-1^{1}), 3.744 (s, OCH_3). **22**: $[\alpha]_D$ +38.9° (c 1.6); δ_H 5.202 (d, 7.9 Hz, H-1^{2}), 5.160 (d, 8.5 Hz, H-1^{9}), 4.972 (d, 8.6 Hz, H-1^{1}). **23**: $[\alpha]_D$ +40.3° (c 0.8); δ_H 5.218 (dd, 1.8, 3.3 Hz, H-$2^{4'}$), 5.188 (d, 8.6 Hz, H-1^{9}), 5.138 (d, 7.6 Hz, H-1^{2}), 4.939 (d, 8.2 Hz, H-1^{1}), 4.871 (d, 1.5 Hz, H-$1^{4'}$); δ_C 101.4 (C-1^{3}), 99.0 (C-$1^{4'}$), 97.3 and 96.9 (1:2, C-$1^{1,2,9}$). **24**: $[\alpha]_D$ +22.5° (c 1.0); δ_H 5.873 (dd, 1.8, 3.1 Hz, H-2^{4}), 5.320 (s, H-$2^{4'}$), 5.180 and 5.146 (2d, 8.2 Hz, H-$1^{2,9}$), 5.154 (d, 1.5 Hz, H-1^{4}), 4.937 (d, 7.9 Hz, H-1^{1}), 4.929 (s, H-$1^{4'}$); δ_C 101.1 (C-1^{3}), 100.3 (C-1^{4}), 98.4 (C-$1^{4'}$), 97.4, 96.9 and 96.6 (C-$1^{1,2,9}$). **25**: $[\alpha]_D$ +13.5° (c 0.5); δ_H 5.873 (dd, 1.8, 3.1 Hz, H-2^{4}), 5.329 (s, H-$2^{4'}$), 5.193, 5.188 (2d, 8.2 Hz, H-$1^{2,9}$), 5.152 (d, 1.8 Hz, H-1^{4}), 4.951 (d, 8.2 Hz, H-1^{1}), 4.950 (d, 1.5 Hz, H-$1^{4'}$). **26**: $[\alpha]_D$ +10.1° (c 0.2), δ_H 5.843 (dd, 1.8, 3.1 Hz, H-2^{4}), 5.332 (d, 8.2 Hz, H-1^{2}), 5.324 (s, H-$2^{4'}$), 5.181 (d, 8.2 Hz, H-1^{9}), 5.158 (d, 1.5 Hz, H-1^{4}), 4.954 (d, 1.5 Hz, H-$1^{4'}$), 4.899 (d, 7.9 Hz, H-1^{1}). **27**: δ_H 5.765 (dd, 1.5, 3.5 Hz, H-2^{4}), 5.327 (dd, 1.5, 3.5 Hz, H-$2^{4'}$), 5.280 (d, 1.5 Hz, H-1^{4}), 2.181, 1.954, 1.904, 1.769, 1.589 (5s, Ac x 5), 0.902 (d, 6.4 Hz, H-6^{F}).
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