A Convergent and Stereocontrolled Synthetic Route to the Core Pentasaccharide Structure of Asparagine-Linked Glycoproteins

Akihito Dan, Yukishige Ito,* and Tomoya Ogawa*,†

The Institute of Physical and Chemical Research (RIKEN), 2-1 Hirosawa, Wako-shi, Saitama 351-01, Japan

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Asparagine (Asn)-linked oligosaccharides occur widely to constitute a major class of glycoprotein oligosaccharides and are known to play numerous important biological roles.[1] These oligosaccharides are usually divided into three classes, namely high-mannose type, complex type, and hybrid type, based on the differences in their outer carbohydrate sequences. Each of these classes, in turn, consists of a range of diverse structures. Yet, in spite of these structural variations, all members of the Asn-linked oligosaccharide family contain a common pentasaccharide "core" structure which is depicted in Figure 1 as a partial structure of a typical complex type glycan chain. Described herein is a convergent and highly stereoselective synthesis of this pentasaccharide sequence (**1a**, Figure 1).

Although extensive efforts have been devoted to the synthesis of Asn-linked oligosaccharides, a general and stereoselective synthetic route has yet to be developed.[2] Among the several unresolved problems that remain, the most synthetically challenging by far is the construction of a β-glycosidic linkage between mannose (Man) and *N*-acetylglucosamine (GlcNAc) residues (Figure 1, underlined). To address this problem, numerous intriguing approaches have been investigated.[3] Among these, an intramolecular aglycon delivery method[3h] introduced by Barresi and Hindsgaul and later by Stork and Kim appears to be the most rational one. As an extension of this concept, we have recently reported the use of a *p*-methoxybenzyl (PMB) group at the *C*-2 position as the stereocontrolling element[4] (Scheme 1). With application of this methodology to the synthesis of a variety of glycoprotein oligosaccharides in mind, we designed the

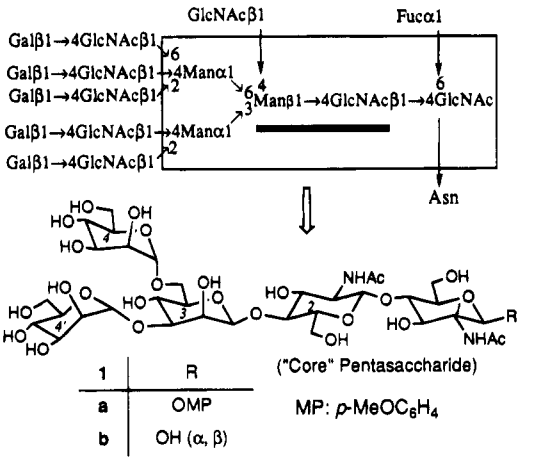
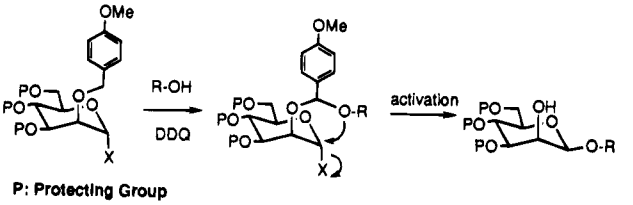
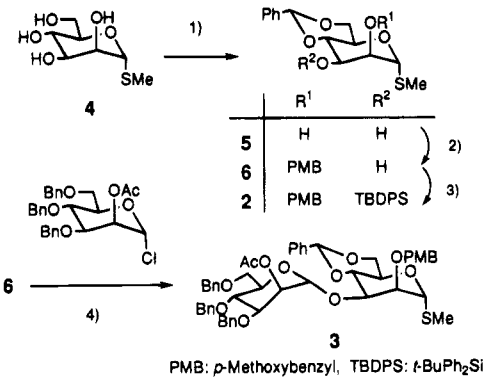


Figure 1. Structure of complex-type Asn-linked oligosaccharide. Boxed part corresponds to the "core" pentasaccharide.

Scheme 1



Scheme 2[a]



[a] Conditions: (1) $PhCH(OMe)_2$, CSA/DMF, 77%; (2) PMB-Cl, Bu_4NHSO_4/CH_2Cl_2–aqueous NaOH,[7] reflux 50% (+ 15% regioisomer); (3) $^tBuPh_2SiCl$, imidazole/DMF, 73%; (4) AgOTf, DMBP, MS4A/CH_2Cl_2, 81%.

selectively protected mono- (**2**) and dimannosyl (**3**) thioglycosides as mannosyl donors.

Syntheses of **2**[5] and **3**[5] were performed starting from the known methyl thiomannoside **4**[6] as shown in Scheme 2. As glycosyl acceptors, monosaccharide **7**[8] and disaccharide **8**[9] were investigated.

The coupling of **2**/**3** with **7**/**8** was performed as follows (Scheme 3). Initial treatments with DDQ, under anhydrous conditions, afforded the mixed acetals **11a–d** quite

† Alternative address: Graduate School of Agriculture and Life Science, University of Tokyo, Yayoi, Bunkyo-ku, Tokyo 113, Japan.

(1) Recent reviews: Kobata, A. *Acc. Chem. Res.* **1993**, *26*, 319. Varki, A. *Glycobiology* **1993**, *3*, 97. Cummings, R. D. In *Glycoconjugates Composition, Structure, and Function*; Allen, H. J., Kisalius, E. C., Eds.; Marcel Dekker: New York, 1992; p 333. Elbein, A. D. *Trends Biotech.* **1991**, *9*, 346. Paulson, J. C. *Trends Biochem. Sci.* **1989**, *14*, 272.

(2) For leading references, see: Yamazaki, F.; Kitajima, T.; Nukada, T.; Ito, Y.; Ogawa, T. *Carbohydr. Res.* **1990**, *201*, 15. Unverzagt, C. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 1102. Merritt, J. R.; Naisang, E.; Fraser-Reid, B. *J. Org. Chem.* **1994**, *59*, 4443. Recent reviews: Kunz, H. *Angew. Chem., Int. Ed. Engl.* **1987**, *26*, 294. Paulsen, H. *Angew. Chem., Int. Ed. Engl.* **1990**, *29*, 823.

(3) (a) Paulsen, H.; Lockhoff, O. *Chem. Ber.* **1981**, *114*, 3102. Paulsen, H.; Lebuhn, R.; Lockhoff, O. *Carbohydr. Res.* **1982**, *103*, C7. (b) Garegg, P. J.; Ossowski, P. *Acta Chem. Scand. Ser. B* **1983**, *37*, 249. (c) van Boeckel, C. A. A.; Beetz, T.; van Aelst, S. F. *Tetrahedron* **1984**, *40*, 4097. van Boeckel, C. A. A.; Beetz, T. *Recl. Trav. Chim. Pays-Bas* **1985**, *104*, 171. (d) Lichtenthaler, F. W.; Schneider-Adams, T. *J. Org. Chem.* **1994**, *59*, 6728. Lichtenthaler, F. W.; Schneider-Adams, T.; Immel, S. *J. Org. Chem.* **1994**, *59*, 6735. (e) Augé, C.; Warren, C. D.; Jeanloz, R. W.; Kiso, M.; Anderson, L. *Carbohydr. Res.* **1980**, *82*, 85. (f) David, S.; Malleron, A.; Dini, C. *Carbohydr. Res.* **1989**, *188*, 193. Alais, J.; David, S. *Ibid.* **1990**, *201*, 69. (g) Kunz, H.; Günther, W. *Angew. Chem., Int. Ed. Engl.* **1988**, *27*, 1086. (h) Barresi, F.; Hindsgaul, O. *Can. J. Chem.* **1994**, *72*, 1447. Barresi, F.; Hindsgaul, O. *J. Am. Chem. Soc.* **1991**, *113*, 9376. Barresi, F.; Hindsgaul, O. *Synlett* **1992**, 759. Stork, G.; Kim, G. *J. Am. Chem. Soc.* **1992**, *114*, 1087.

(4) Ito, Y.; Ogawa, T. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 1765.

(5) Experimental procedures for the preparation of key compounds are given in the supplementary material.

(6) Ogawa, T.; Matsui, M. *Carbohydr. Res.* **1977**, *54*, C17.

(7) Garegg, P. J.; Kvarnström, I.; Niklasson, A.; Niklasson, G.; Svensson, S. C. T. *J. Carbohydr. Chem.* **1993**, *12*, 933.

(8) Nakano, T.; Ito, Y.; Ogawa, T. *Carbohydr. Res.* **1993**, *243*, 43.

(9) Kuyama, H.; Nakahara, Y.; Nukada, T.; Ito, Y.; Nakahara, Y.; Ogawa, T. *Carbohydr. Res.* **1993**, *243*, C1.

(10) The formation of mixed acetal was confirmed for **11d**, which was isolated in 88% yield. For details, see the supporting information.

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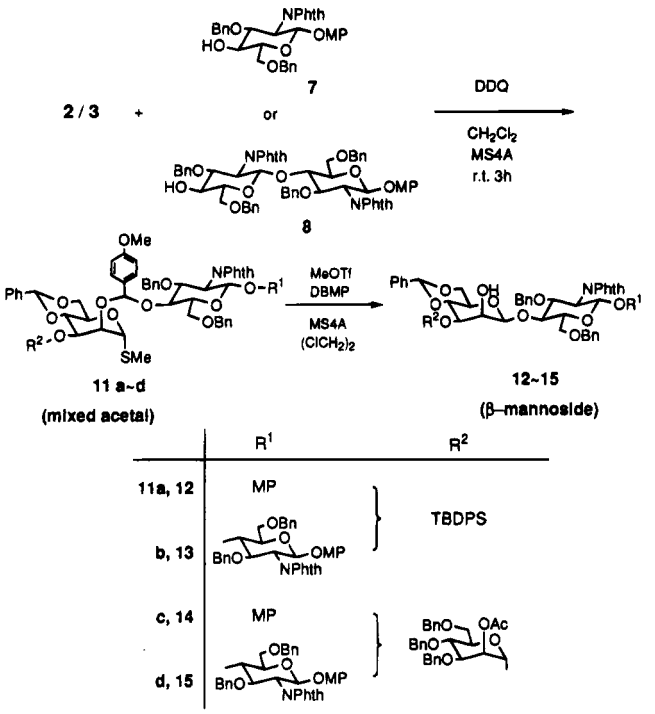
Scheme 3



smoothly.[10] Subsequent activation of the anomeric position was achieved most efficiently using methyl triflate (MeOTf)[11] in the presence of 2,6-di-*tert*-butyl-4-methylpyridine (DBMP) and molecular sieves (MS) 4A.[12] As summarized in Table 1, all combinations afforded the β-linked structures **12**–**15**[5] as the only isolable glycosylated products.

It should be noted that the choice of protecting groups for these fragments was made in order to facilitate elongation of these glycan chains into any of the various branching patterns found in Asn-linked oligosaccharides.

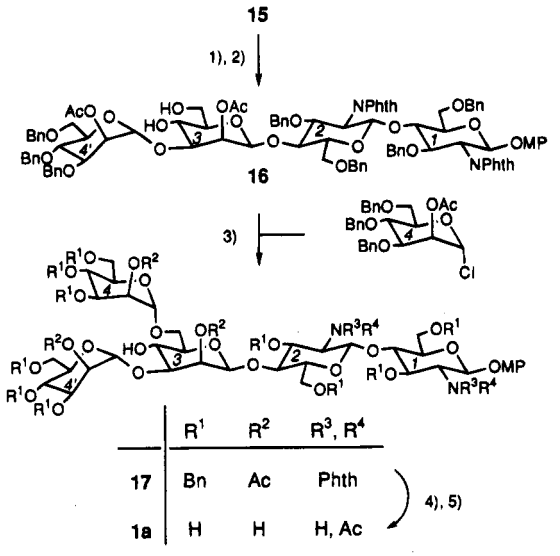
To demonstrate the utility of the β-mannoside products thus obtained, tetrasaccharide **15** was converted into pentasaccharide **1a**, which represents the core structure of Asn-linked oligosaccharides (Scheme 4). The ^{1}H-NMR data of **1a**[14] were in good agreement with that reported for reducing pentasaccharide (**1b**) obtained from natural sources.[15]

Table 1. Results of β-Mannosidation Reactions

entry	thioglycoside (equiv)	acceptor	11	T (°C)/ time[a]	product	yield[b] (%)
1	**2** (1.1)	**7**	**a**	rt/20 h	**12**	60
2	**2** (1.4)	**8**	**b**	rt/2 d	**13**	60
3	**3** (1.4)	**7**	**c**	40 °C/14 h	**14**	53
4	**3** (1.3)	**8**	**d**[c]	40 °C/4 d	**15**	49

[a] For conversion of **11** into **12**–**15**. [b] In entries 2 and 4, 11% and 23% of acceptor was recovered, respectively. [c] Isolated in 88% yield.

Scheme 4[a]



[a] Conditions: (1) Ac_2O, Et_3N, 4-DMAP/MeCN, 99%; (2) (±)-camphorsulfonic acid/MeOH, 60 °C, 75%; (3) AgOTf, MS4A/CH_2Cl_2, 79%; (4) $NH_2CH_2CH_2NH_2$/BuOH,[13] 90 °C, then Ac_2O/MeOH, 0 °C, 83%; (5) H_2, Pd–C/80% MeOH, 94%.

Further extension of this method to various naturally-occurring Asn-linked oligosaccharides is currently under investigation.

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Supporting Information Available: NMR spectral data and experimental procedures for the preparation of compounds **1a**, **2**, **3**, **6**, and **12**–**17** and copies of ^{1}H- and ^{13}C-NMR spectra of compound **1a** (13 pages).

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(11) Lönn, H. *J. Carbohydr. Chem.* **1987**, *6*, 301.

(12) A typical experimental procedure is given for the preparation of compound **12**: A solution of compounds **2** (58.1 mg, 0.0884 mmol) and **7** (46.3 mg, 0.0777 mmol) in CH_2Cl_2 (2 mL) was added dropwise to a stirred mixture of DDQ (23.2 mg, 0.102 mmol) and MS4A (0.2 g) in CH_2Cl_2 at 0 °C. The mixture was stirred at room temperature for 2.5 h, quenched by stirring with a solution of ascorbic acid (0.7%)–citric acid (1.3%)–NaOH (0.9%) in water (ca. 2 mL), and filtered through Celite. The filtrate was washed successively with aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, and evaporated *in vacuo*. The residue was exposed to high vacuum for 1 h to give crude **11a**, which was mixed with DBMP (88 mg, 0.43 mmol) and MS4A (0.2 g) in CH_2Cl_2 (2.5 mL). To the ice–water-cooled mixture was added MeOTf (44 μL, 0.39 mmol), and the whole was stirred at room temperature for 20 h. Triethylamine (0.3 mL) was added, and the mixture was stirred for 10 min, diluted with AcOEt, and filtered through Celite. The filtrate was washed with water and brine, successively, dried over Na_2SO_4, and evaporated *in vacuo*. The residue was purified by a column of Bio-Beads S-X4 (BioRad) in toluene and then by silica gel column chromatography (toluene–AcOEt 1:0–12:1) to afford 50.5 mg (60%) of compound **12**.

(13) Kanie, O.; Crawley, S. C.; Palcic, M. M.; Hindsgaul, O. *Carbohydr. Res.* **1993**, *243*, 139.

(14) **1a**: ^{1}H-NMR (D_2O, 400 MHz); δ 7.03–6.09 (m, 4H), 5.09 (1H, d, J = 1.0 Hz), 5.02 (1H, d, J = 8.3 Hz), 4.90 (1H, d, J = 1.5 Hz), 4.77 (1H, s), 4.62 (1H, d, J = 7.8 Hz), 3.79 (3H, s), 2.07 and 2.02 (3H × 2, s); ^{13}C-NMR (D_2O); δ 104.26, 103.09, 102.14, 102.10, and 101.33; R_f 0.57 (*n*-BuOH–MeOH–H_2O–AcOH 4:2:2:1).

(15) Davis, J. T.; Hirani, S.; Bartlett, C.; Reid, B. R. *J. Biol. Chem.* **1994**, *269*, 3331. For the first chemical synthesis of **1b**, see: Paulsen, H.; Lebuhn, R. *Carbohydr. Res.* **1984**, *130*, 85.