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Published on Web 02/28/2003

Synthesis of Monoglucosylated High-Mannose-Type Dodecasaccharide, a Putative Ligand for Molecular Chaperone, Calnexin, and Calreticurin

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Addition of the asparagine (Asn)-linked (*N*-linked) glycan chain is a prominent posttranslational modification of proteins.[1] Because of the discoveries of critical roles of high-mannose-type oligosaccharides in various aspects of protein quality control, their biological significance is attracting renewed attention.[2] Particularly important in this respect is the calnexin/calreticulin-glucosyltransferase cycle. Calnexin (CNX) and carleticulin (CRT) are homologous chaperones that reside in the lumen of endoplasmic reticulum (ER). Recent investigations have clarified that the interaction of glycoprotein with CNX/CRT is primarily mediated by the terminally monoglucosylated high-mannose-type glycan chain, most probably $Glc_1Man_9GlcNAc_2$ (**2a**).[3,4] On the other hand, UDP-glucose:glycoprotein glucosyltransferase (UGGT) is believed to function as a "folding sensor", which detects incompletely folded glycoproteins carrying $Man_9GlcNAc_2$ (**1a**) and reglucosylates them back to **2a**.[5] However, the precise role of the glycan chain in chaperone recognition is still controversial.[6] We report herein the first chemical syntheses of the putative ligand of CNX/CRT, $Glc_1Man_9GlcNAc_2$ (**2b**) (Figure 1). Additionally, the stereoisomeric dodecasaccharide carrying β-linked Glc (**3**) was synthesized. Furthermore, the first NMR-based evidence for the specific binding of CRT to α-$Glc_1Man_9GlcNAc_2$ was obtained.

Undecasaccharides **2b** and **3** were retrosynthetically disconnected to fragments **6**, **7**, and **8** (Figure 1). For the construction of β-mannoside containing fragment **6**, *p*-methoxybenzyl assisted intramolecular aglycon delivery[7] was used as the key reaction (Scheme 1). Thus, β-mannosylation of glucosamine derivative **10**[8] using **9** as a donor[7a] was performed by way of the mixed acetal **11**, according to the standard protocol to afford disaccharide **12a** in 82% yield as a single stereoisomer. Corresponding acetate **12b** was then used as the glycosyl donor and coupled with **13**[9] to give **14** which was converted to diol **6**. Tri- and pentamannoside fragments **7** and **8** were synthesized from **15**[10] and **19**,[11] respectively (Scheme 1).

With all fragments in hand, the undecasaccharide skeleton was assembled as depicted in Scheme 2. Coupling of **6** with **7** was achieved by the action of MeOTf[12] to afford 3-*O*-glycosylated hexasaccharide **22a**, together with its regioisomer in 77 and 13% yield, respectively. Subsequent acetylation and removal of the cyclohexylidene group afforded diol **22c**, which in turn was reacted with pentasaccharide donor **8** to give undecasaccharide **23a** in 87% yield which was converted to acetate **23b**. Removal of the TBDPS group was performed under high-pressure conditions as described before[13] to give **23c** in 86% yield. For the incorporation of α-linked glucose residue, thioglucoside **4** was proven to be highly satisfactory as the glycosyl donor and provided desired dodecasaccharide **24** as a single isomer in 85% yield.[14] The stereoisomeric dodecasaccharide carrying β-linked glucose residue **25** was synthesized using glycosyl donor **5** in 81% yield. Finally, complete deprotection

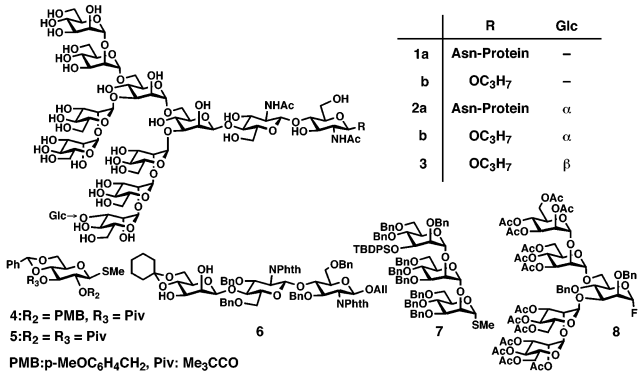
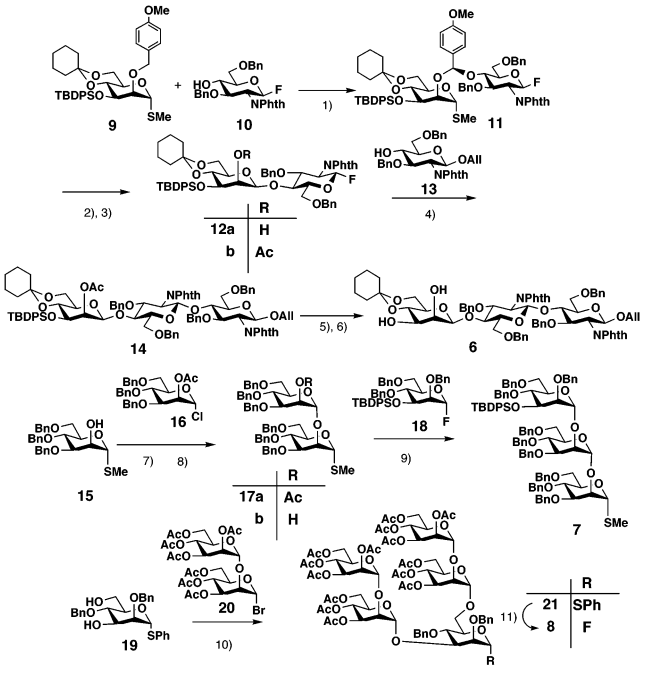


Figure 1. Structures of high-mannose-type glycans.

Scheme 1. Preparation of Oligosaccharide Blocks[a]



[a] Reagents and yields: (1) DDQ; (2) MeOTf, DTBMP, $ClCH_2CH_2Cl$, 83% (two steps); (3) Ac_2O, pyridine, DMAP, 96%; (4) Cp_2HfCl_2, AgOTf, CH_2Cl_2, 85%; (5) TBAF/AcOH, DMF, 82%; (6) NaOMe/MeOH, 75%; (7) AgOTf, CH_2Cl_2, 81%; (8) NaOMe/MeOH, quant.; (9) Cp_2HfCl_2, AgOTf, CH_2Cl_2, 91%; (10) AgOTf, CH_2Cl_2, 72%; (11) NBS, DAST, 85%.

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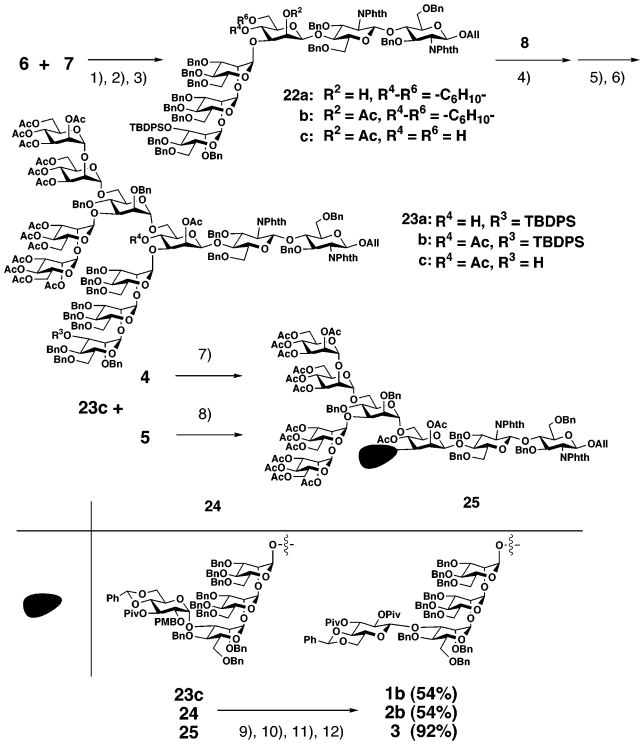
10.1021/ja021288q CCC: $25.00

Scheme 2. Convergent Synthesis of Undecasaccharide and Introduction of Glucose Residue[a]



[a] Reagents and yields: (1) MeOTf, 77%; (2) Ac_2O, pyridine, DMAP; (3) *p*-TosOH, 56% (two steps); (4) Cp_2HfCl_2, AgOTf, toluene, 87%; (5) Ac_2O, pyridine, DMAP, 98%; (6) 10% HF/pyridine, DMF, 1 GPa, 86%; (7) MeOTf, $ClCH_2CH_2Cl$/cyclohexane, 85%; (8) MeOTf, ClH_2CH_2Cl, 81%; (9) ethylenediamine, *n*-BuOH; (10) Ac_2O, pyridine, DMAP; (11) $Pd(OH)_2$, H_2, 80% AcOH; (12) NaOMe/MeOH.

of **23c**, **24**, and **25** afforded $Man_9GlcNAc_2$ (**1b**),[15] $Glc_1Man_9GlcNAc_2$ (**2b**), and β-$Glc_1Man_9GlcNAc_2$ (**3**), respectively (Scheme 2).

Synthetic dodecasaccharide **2b** was subjected to ^{1}H NMR measurements to observe the specific interaction with CRT. In the presence of recombinant CRT,[16] peak heights of all anomeric signals decreased in proportion with the amount of CRT, suggesting that **2b** binds tightly with CRT under these conditions (Figure 2A).[17] These peaks eventually disappeared after the addition of ~1 equiv of CRT. That the CRT−ligand interaction is specific to the fine structure of the oligosaccharide was supported by a similar measurement using a 1:1 mixture of **2b** and its stereoisomer **3**. In the presence of 0.5 equiv (with respect to the mixture of **2b** and **3**) of CRT, peaks derived from α-linked **2b** were strongly suppressed, while those from **3** were barely affected under these conditions (Figure 2B).

In conclusion, convergent and stereoselective synthetic routes to $Man_9GlcNAc_2$ (**1b**), α-$Glc_1M_9GlcNAc_2$ (**2b**), and its stereoisomer (**3**) were established. Using ^{1}H NMR, we observed the interaction of **2b** with CRT. A more systematic study is in progress and will be reported in due course.

Acknowledgment. We thank Dr. Teiji Chihara for the operation of the high-pressure equipment, Prof. Shunji Natori for providing the GST-CRT construct, and Ms. Akemi Takahashi for technical assistance. Financial support from the Mizutani Foundation for Glycoscience and Ministry of Education, Culture, Sports, Science and Technology [Grant-in-Aid for Scientific Research (B) No. 13480191] is acknowledged.

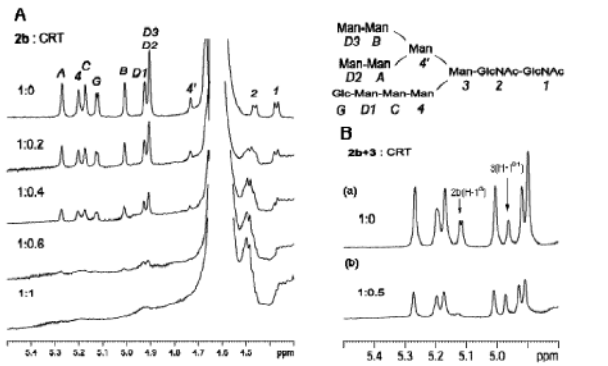


Figure 2. (A) Anomeric region of 1D ^{1}H NMR spectra acquired of α-$GlcMan_9GN_2$ (**2b**) in ^2H_2O, 10 mM Tris-HCl buffer, 10 mM $CaCl_2$ at pH 7.3. CRT was added to the sample stepwise; the final ratio of **2b** and CRT was 1:1. (B) Anomeric region of 1D ^{1}H NMR spectra acquired of **2b** + **3** (1:1) without (a) and with (b) 0.5 equiv of CRT.

Supporting Information Available: Preparative methods and ^{1}H and ^{13}C NMR spectra of new compounds and procedure for the expression of CRT (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Dwek, R. A. *Chem. Rev.* **1996**, *96*, 683−720.
(2) (a) High, S. H.; Lecomte, F. J. L.; Russel, S. J.; Abell, B. M.; Oliver, J. D. *FEBS Lett.* **2000**, *476*, 38−41. (b) Cabral, C. M.; Liu, Y.; Sifers, R. N. *Trends Biochem. Res.* **2001**, *26*, 619−624. (c) Braakman, I. *EMBO Rep.* **2001**, *2*, 666−668.
(3) (a) Helenius, A.; Aebi, M. *Science* **2001**, *291*, 2364−2369. (b) Spiro, R. G. *J. Biol. Chem.* **2000**, *46*, 35657−35660. (c) Petrascu, S. M.; Branza-Nichita, N.; Negroiu, G.; Petrescu, A. J.; Dwek, R. A. *Biochemistry* **2000**, *39*, 5229−5237. For the crystal structure of CNX, see: (d) Scrag, J. D.; Bergeron, J. J. M.; Li, Y.; Borisova, S.; Hahn, M.; Thomas, D. Y.; Cygler, M. *Mol. Cell* **2001**, *8*, 633−644.
(4) (a) Leach, M. R.; Cohen-Doyle, M. F.; Thomas, D. Y.; Williams, D. B. *J. Biol. Chem.* **2002**, *277*, 29686−29697. (b) Patil, A. P.; Thomas, C. J.; Surolia, A. *J. Biol. Chem.* **2000**, *275*, 24348−24356.
(5) Trombetta, E. S.; Helenius, A. *J. Cell Biol.* **2000**, *148*, 1123−1129.
(6) Ihara, Y.; Cohen-Doyle, M. F.; Saito, Y.; Williams, D. B. *Mol. Cell* **1999**, *4*, 331−341.
(7) (a) Ito, Y.; Ohnishi, Y.; Ogawa, T.; Nakahara, Y. *Synlett* **1998**, 1102−1104. (b) Seifert, J.; Lergenmüller, M.; Ito, Y. *Angew. Chem., Int. Ed.* **2000**, *39*, 531−534.
(8) Kanie, O.; Ito, Y.; Ogawa, T. *J. Am. Chem. Soc.* **1994**, *116*, 12073−12074.
(9) Ogawa, T.; Kitajima, T.; Nukada, T. *Carbohydr. Res.* **1983**, *123*, C5−C7.
(10) Ito, Y.; Kanie, O.; Ogawa, T. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 2510−2512.
(11) Matsuo, I.; Isomura, M.; Miyazaki, T.; Sakakibara, T.; Ajisaka, K. *Carbohydr. Res.* **1998**, *305*, 401−413.
(12) Lönn, H. *J. Carbohydr. Chem.* **1987**, *6*, 301−306.
(13) Matsuo, I.; Wada, M.; Ito, Y. *Tetrahedron Lett.* **2002**, *43*, 3273−3275.
(14) Reaction using tetra-*O*-benzyl thioglycoside under similar conditions afforded a mixture of α- and β-isomers (α:β = 3:1).
(15) This compound was demonstrated to bind with F-box protein Fbx2, a constituent of ubiquitin ligase: Yoshida, Y.; Chiba, T.; Tokunaga, F.; Kawasaki, H.; Iwai, K.; Suzuki, T.; Ito, Y.; Matsuoka, K.; Yoshida, M.; Tanaka, K.; Tai, T. *Nature* **2002**, *418*, 438−442.
(16) For the expression of CRT, see Supporting Information.
(17) Siriwardena, A. H.; Tian, F.; Noble, S.; Prestegard, J. H. *Angew. Chem., Int. Ed.* **2002**, *41*, 3454−3457.

JA021288Q