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Rapid Communication

Synthesis of an octamannosyled glycan chain, the key oligosaccharide structure in ER-associated degradation

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Abstract

The high-mannose type decasaccharide ($Man_8GlcNAc_2$), the proposed ligand of ER residing mannosidase-like proteins (MLP), and its monoglycosylated homologue (α-$Glc_1Man_8GlcNAc_2$) were synthesized. The oligosaccharide assembly was performed in a convergent and stereoselective manner, using three oligosaccharide components, a core trisaccharide having a β-mannoside bond, a liner mannotriose, and a branched mannotetraose.

Keywords: Synthesis; Octamannosyled glycan chain; Oligosaccharide structures

Oligosaccharide parts of glycoproteins play critical roles in numerous biological events.[1] In particular, important roles of asparagine (Asn)-linked oligosaccharides in glycoprotein quality control have attracted recent attention (Fig. 1). The central role of molecular chaperones, calnexin (CNX) and calreticulin (CRT), has been well-documented.[2,3]

These compounds share a unique lectin property and recognize monoglucosylated dodecasaccharide ($Glc_1Man_9GlcNAc_2$, G1M9) as a primary ligand, which is formed by the stepwise removal of two Glc residues from the tetradecasaccharide ($Glc_3Man_9GlcNAc_2$, G3M9) that is introduced to nascent polypeptides as a common precursor of all Asn-linked glycans. After removal of the innermost glucose by glucosidase II, correctly folded proteins are transported to the Golgi apparatus for further processing, while misfolded ones are reglucosylated back to G1M9 by the action of UDP-glucose:glycoprotein glucosyltransferase (UGGT).[4] On the other hand, 'hopelessly misfolded' glycoproteins are delivered to the degradation pathway, the so-called ER-associated degradation (ERAD).[5] Mannosidase-like proteins (MLPs), EDEM discovered from mammalian cells[6] and its yeast counterpart Mn11p/Htm1p,[7] are considered to have a lectin property and recognize glycoproteins having M8 oligosaccharides ($Man_8GlcNAc_2$, B-isomer, **1a**), which are formed by the action of ER mannosidase-I from M9.[8] Glycoproteins directed to ERAD are dislocated to the cytosol,[9] ubiquitinated,[10] deglycosylated[11] and degraded by the proteasome.[12]

Although the proposed role of MLPs seems reasonable in a teleological sense, concrete evidence of their M8 specific lectin property has been lacking. In addition, it is not clear if monoglucosylated M8 (G1M8, **2a**) is involved in the CNX/CRT cycle and/or MLP-mediated ERAD. In order to gain a clear understanding of the molecular mechanism of glycoprotein quality control, access to the structurally defined oligosaccharide is of critical importance. Recently, we achieved the first chemical synthesis of monoglucosylated dodecasaccharide (G1M9), which is a putative ligand of CNX/CRT.[13] As part of our program on the systematic synthesis of high-mannose type oligosaccharides related to protein quality control, we report herein the syntheses of the proposed ligand of MLPs, $Man_8GlcNAc_2$ (M8, **1b**) and its monoglucosylated homologue $Glc_1Man_8GlcNAc_2$ (G1M8, **2b**).

For the convergent synthesis of target oligosaccharides, fragments corresponding to $Man_1GlcNAc_2$ (**4**),

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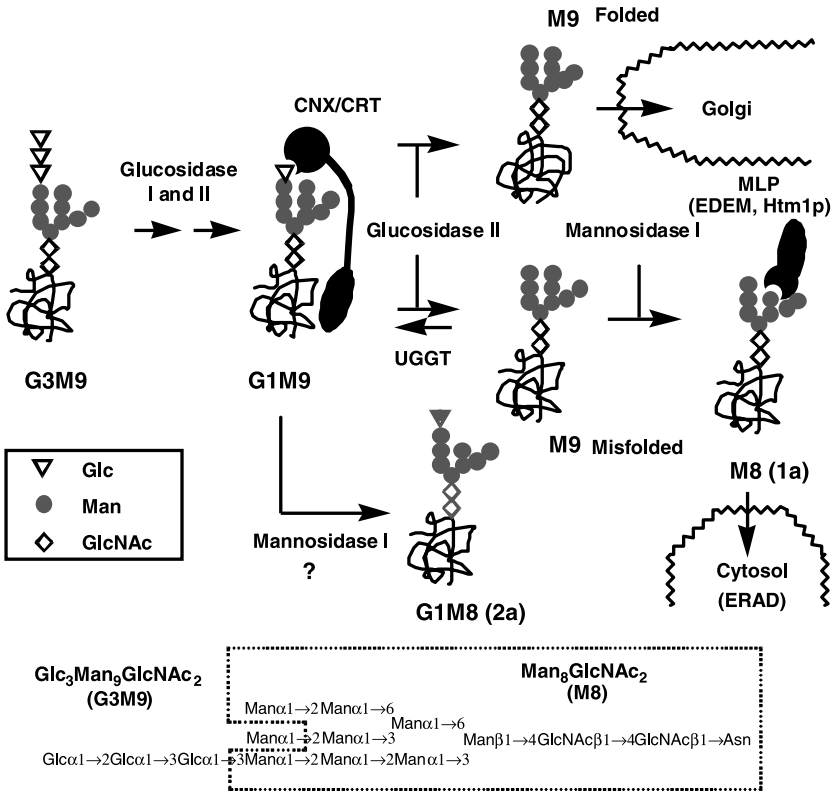


Fig. 1. Glycoprotein processing and quality control in ER.

Man_4 (**5**) and Man_3 (**6**) were designed (Fig. 2). Fragment **4** that contains a β-mannoside linkage was to be constructed by *p*-methoxybenzyl (PMB) assisted intramolecular aglycon delivery (IAD)[14] as the key reaction, while one-pot sequential glycosylation strategy was adopted for the preparation of the tetrasaccharide fragment **5** (vide infra). Preparation of trimannoside **6** was reported previously.[13]

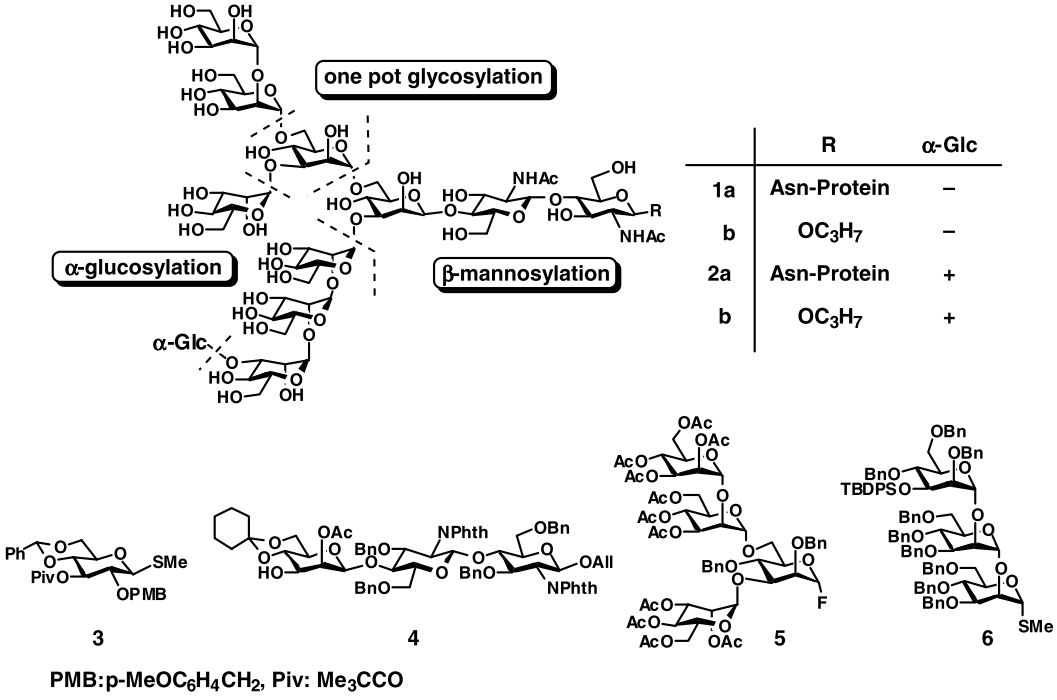
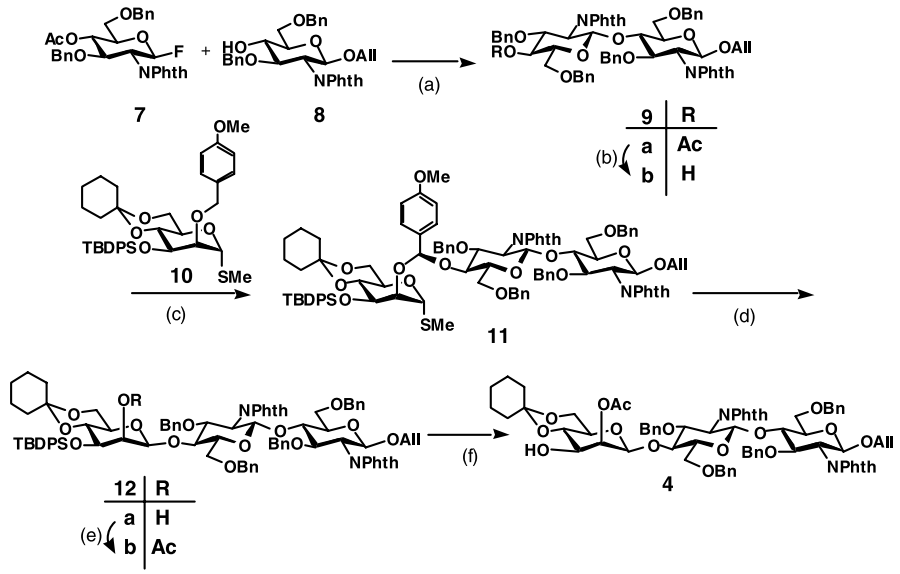


Fig. 2. Synthetic plan for high-mannose type undecasaccharide.

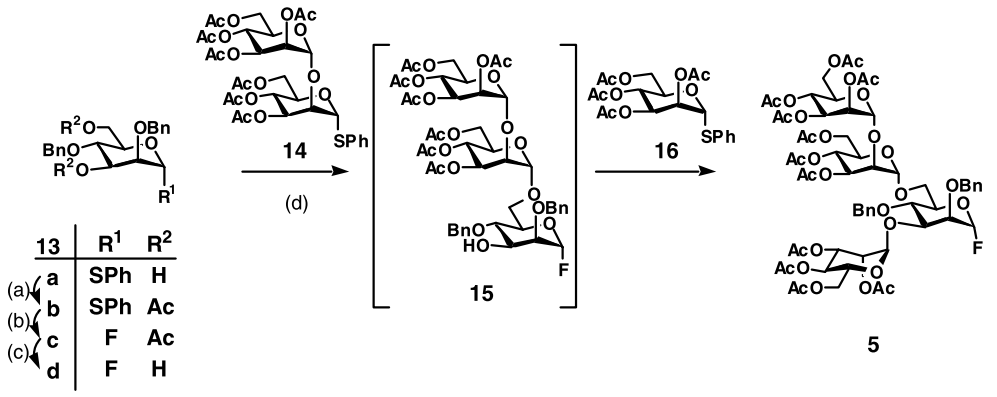


Scheme 1. Reagents and conditions: (a) Cp_2HfCl_2, AgOTf, 4A MS, CH_2Cl_2, −45 °C, 2 h. (b) H_2O_2, LiOH, THF, 0 °C, 2 h, 64% (two steps). (c) DDQ, 4A MS, CH_2Cl_2, rt, 2 h. (d) MeOTf, DTBMP, 4A MS, $ClCH_2CH_2Cl$, 45 °C, 12 h, 74% (two steps). (e) Ac_2O, pyridine, DMAP, rt, 12 h, quant. (f) HF–pyridine, DMF, 1 GPa, rt, 12 h, 88%.

As a precursor of fragment **4**, previously reported **12b**[13] was prepared in a slightly modified manner as shown in Scheme 1. The coupling of compounds **7**[15] and **8**[16] was performed by using Suzuki's protocol[17] to give **9a** that was deacetylated under controlled conditions (H_2O_2, LiOH–THF).[18] Resultant **9b**, obtained in 64% yield (two steps), was then subjected to β-mannosylation using 2-*O*-PMB-equipped thioglycoside **10** as a donor.[14] The reaction was performed according to our standard two-step procedure; DDQ-mediated formation of mixed acetal **11**, and subsequent IAD promoted by MeOTf afforded trisaccharide **12a** in 74% yield as a single stereoisomer. Stereochemistry of the mannoside linkage was confirmed to be β by ^{13}C NMR spectroscopy (C-1^{Man}: 99.7 ppm, $^1J_{CH}$ 156 Hz). The product **12a** was converted into corresponding acetate **12b** in quantitative yield. Subsequent desilylation was achieved cleanly under high pressure (1 GPa) with HF–pyridine to give trisaccharide acceptor **4** in 88% yield.[19] By contrast, this particular transformation under standard conditions was problematic. For instance, treatment with TBAF–AcOH resulted in an extensive migration of the acetyl group to afford a mixture of 2-*O*-Ac and 3-*O*-Ac products, while treatment with HF–pyridine under atmospheric pressure resulted in the complete recovery of **12b**.

Synthesis of tetramannoside fragment **5** was carried out as depicted in Scheme 2. It was started with the preparation the glycosyl fluoride **13d** having two hydroxy groups at the C-3 and C-6 positions. The thiophenyl glycoside **13a**[20] was acetylated to give **13b** that was converted to the fluoride **13c** using NBS–DAST.[21] Removal of the acetyl groups proceeded uneventfully under standard conditions to give diol **13d** in 77% yield (three steps). One-pot sequential glycosylation was performed with chemoselective activation[22] of thioglycoside **14** and **16**. The thioglycosides **14** and **16** were synthesized from octaacetylmannobiose[20] and pentaacetylmannose by treatment with thiophenol and TMSOTf, respectively. The first glycosylation with 1.1 equiv of mannobiose donor **14** was initiated in the presence of NIS and 1 equiv of TfOH in dichloromethane at −40 °C. After 1 h, TLC and TOF-MS analyses revealed the predominant formation of monoglycosylated product (presumably C-6 glycosylated trisaccharide **15**). Subsequent addition of the monosaccharide donor **16** to the reaction mixture provided the desired tetrasaccharide fragment **5** in 48% yield (pentasaccharide derivative was obtained in 21% yield). The stereochemistries of the newly formed α-mannoside linkages were confirmed by their $^1J_{CH}$ values (174 and 176 Hz).

With all fragments in hand, the decasaccharide skeleton was constructed as shown in Scheme 3. Coupling of **4** with **6** was achieved by the action of MeOTf[23] to afford 3-*O*-glycosylated hexasaccharide **17a**, which was isolated as a single isomer in 76% yield. Removal of the cyclohexylidene group afforded diol **17b**, and regioselective glycosylation of 6-OH of the β-mannoside residue with the tetrasaccharide donor **5** gave decasaccharide **18a** that was converted to acetate **18b**. Removal of the TBDPS group was performed under high-pressure conditions as described before[19] to give **18c** in 70% yield. For the incorporation of the α-linked glucose residue, glycosylation with the thioglucoside **3** proceeded satisfactorily as described before[13] and pro-



Scheme 2. Reagents and conditions: (a) Ac_2O, pyridine, rt, 7 h. (b) NBS, DAST, CH_2Cl_2, −10 °C, 12 h. (c) NaOMe, MeOH, 0 °C, 4 h, 77% (three steps). (d) NIS, TfOH, 4A MS, CH_2Cl_2, −40 °C, 1 h, then **16**, −40 °C, 0.5 h, 48% (+21% pentasaccharide).

vided the desired undecasaccharide **19** as a single isomer in 74% yield. Finally, complete deprotection of **18c** and **19** afforded $Man_8GlcNAc_2$ (**1b**) and $Glc_1Man_8GlcNAc_2$ (**2b**), respectively. The ^1H NMR spectrum of **1b** and **2b** were in good agreement with the ones reported for closely related compounds (Fig. 3).[24]

In conclusion, convergent and stereoselective synthetic routes to $Man_8GlcNAc_2$ (**1b**), and α-$Glc_1M_8GlcNAc_2$ (**2b**) were established. By employing high-pressure assisted desilylation and one-pot glycosylation, the target sugar chains were synthesized in a concise manner. These oligosaccharides could be useful as molecular probes to analyze glycoprotein-MLP recognition.

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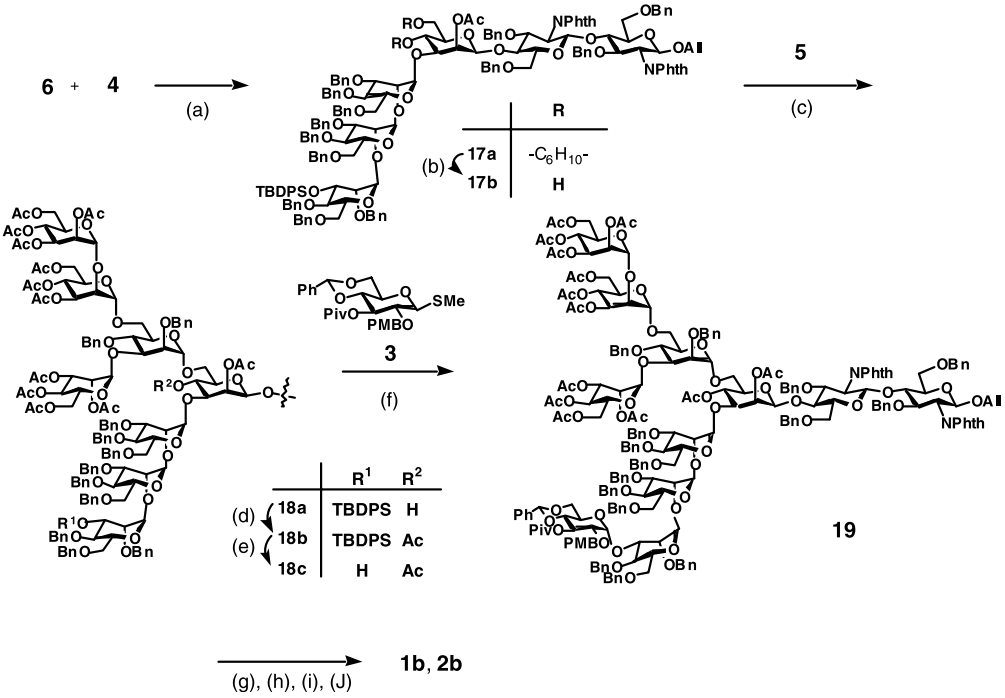

Scheme 3. Reagents and conditions: (a) MeOTf, 4A MS, $ClCH_2CH_2Cl$, 40 °C, 12 h, 76%. (b) *p*-TosOH·H_2O, MeCN, 56%. (c) Cp_2HfCl_2, AgOTf, 4A MS, toluene, −40 to −20 °C, 10 h, 72%. (d) Ac_2O, pyridine, DMAP, rt, 72 h, 82%. (e) 10% HF–pyridine, DMF, 1 GPa, rt, 18 h, 70%. (f) MeOTf, DT BMP, $ClCH_2CH_2Cl$–cyclohexane, 4A MS, 30 °C, 60 h, 74%. (g) ethylenediamine, *n*-BuOH, 70 °C, 48 h. (h) Ac_2O, pyridine, DMAP. (i) $Pd(OH)_2$, H_2, 80% AcOH, rt, 4 days. (j) NaOMe–MeOH, 40 °C, 12 h (**1b**: 87% from **18c**, **2b**: 58% from **19**).

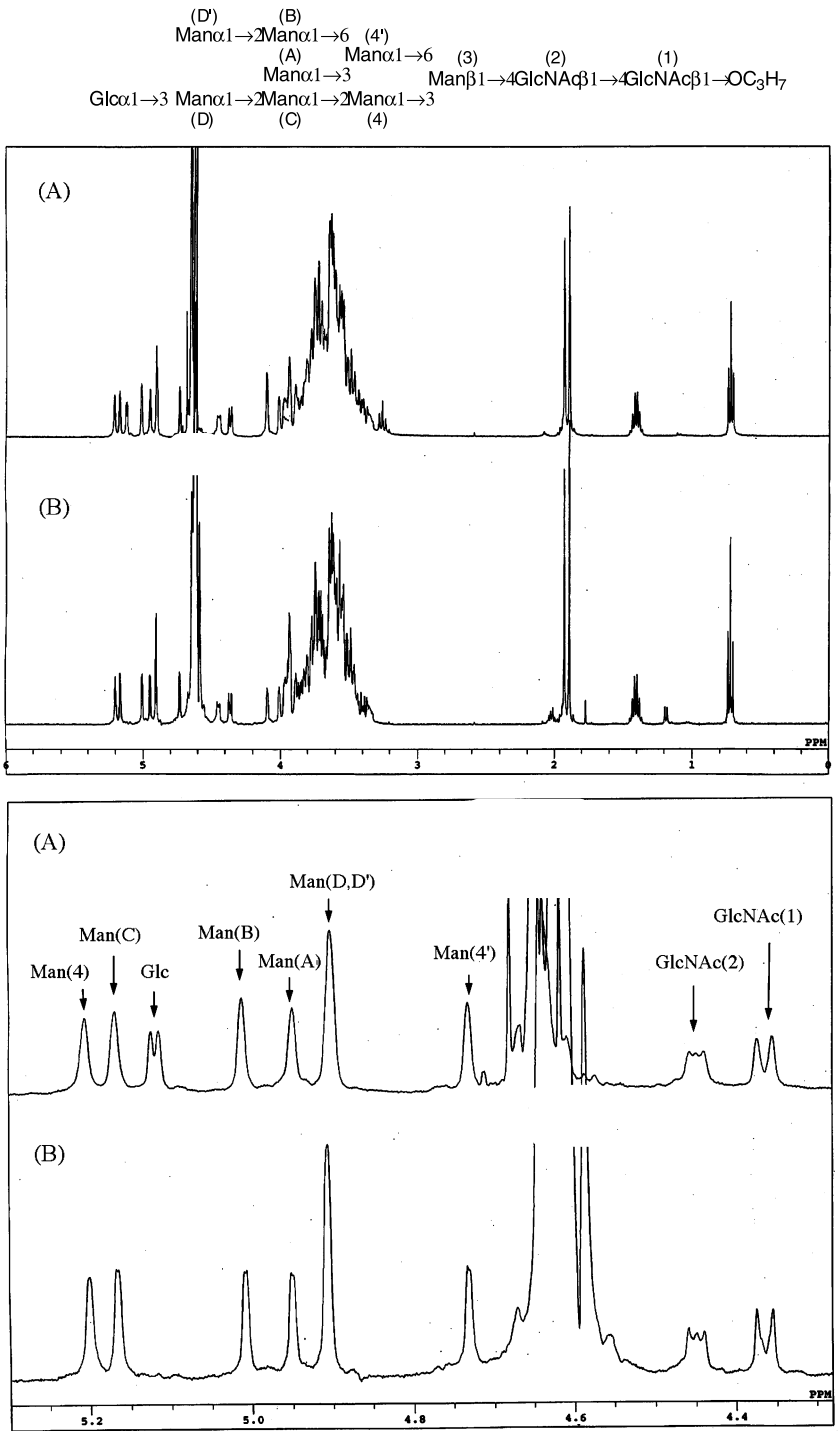

Fig. 3. ^{1}H NMR spectra (400 MHz, D_2O, 23 °C, referenced to HOD adjusted to 4.65 ppm) of undecasaccharide **2b** (A) and decasaccharide **1b** (B).

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References

1. Dwek, R. A. *Chem. Rev.* **1996**, *96*, 683–720.
2. Helenius, A.; Aebi, M. *Science* **2001**, *291*, 2364–2369.
3. Ellgaard, L.; Helenius, A. *Nature Rev. Mol. Cell Biol.* **2003**, *4*, 181–191.
4. Caramelo, J. J.; Castro, O. A.; Alonso, L. G.; de Prat-Gay, G.; Parodi, A. J. *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 86–91.
5. Tsai, B.; Ye, Y.; Rapoport, T. A. *Nature Rev. Mol. Cell Biol.* **2002**, *3*, 246–255.

6. (a) Hosokawa, N.; Wada, I.; Hasegawa, K.; Yorihuzi, T.; Tremblay, L. O.; Herscovics, A.; Nagata, K. *EMBO Rep.* **2001**, *2*, 415–422;
(b) Oda, Y.; Hosokawa, N.; Wada, I.; Nagata, K. *Science* **2003**, *299*, 1394–1397;
(c) Molinari, M.; Calanca, V.; Galli, C.; Lucca, P.; Paganetti, P. *Science* **2003**, *299*, 1397–1400.
7. (a) Jakob, C. A.; Bodmer, D.; Spirig, U.; Bättig, P.; Mardil, A.; Dignard, D.; Bergeron, J. J. M.; Thomas, D. Y.; Aebi, M. *EMBO Rep.* **2001**, *2*, 423–430;
(b) Nakatsukasa, K.; Nishikawa, S.; Hosokawa, N.; Nagatam, K.; Endo, T. *J. Biol. Chem.* **2001**, *276*, 8635–8638.
8. Liu, Y.; Choudhury, P.; Cabra, C. M.; Sifers, R. N. *J. Biol. Chem.* **1999**, *274*, 5861–5867.
9. (a) Braakman, I. *EMBO Rep.* **2001**, *2*, 666–668;
(b) Suzuki, T.; Lennarz, W. J. *Glycobiology* **2002**, *12*, 803–811.
10. 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.
11. Suzuki, T.; Park, H.; Lennarz, W. J. *FASEB J.* **2002**, *16*, 635–641.
12. Glickman, M. H.; Ciechanover, A. *Physiol. Rev.* **2001**, *82*, 373–428.
13. Matsuo, I.; Wada, M.; Manabe, S.; Yamaguchi, Y.; Otake, K.; Kato, K.; Ito, Y. *J. Am. Chem. Soc.* **2003**, *125*, 3402–3403.
14. (a) Ito, Y.; Ohnishi, Y.; Ogawa, T.; Nakahara, Y. *Synlett* **1998**, 1102–1104;
(b) Seifert, J.; Lergenmüller, M.; Ito, Y. *Angew. Chem., Int. Ed. Engl.* **2000**, *39*, 531–534.
15. (a) Unverzagt, C. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 1102–1104;
(b) Kanie, O.; Ito, Y.; Ogawa, T. *J. Am. Chem. Soc.* **1994**, *116*, 12073–12074.
16. El-Sokkary, R. I.; Silwanis, B. A.; Nashed, M. A.; Paulsen, H. *Carbohydr. Res.* **1990**, *203*, 319–323.
17. Suzuki, K.; Maeta, H.; Matsumoto, T. *Tetrahedron Lett.* **1989**, *30*, 4853–4854.
18. (a) Nakano, T.; Ito, Y.; Ogawa, T. *Carbohydr. Res.* **1993**, *243*, 43–69;
(b) Corey, E. J.; Kim, S.; Yoo, S; Nicolaou, K. C.; Melvin, L. S.; Brunelle, P. J.; Flack, J. R.; Trybulsky, E. J.; Lett, R.; Sheldrake, R. W. *J. Am. Chem. Soc.* **1978**, *100*, 4620–4622.
19. Matsuo, I.; Wada, M.; Ito, Y. *Tetrahedron Lett.* **2002**, *43*, 3273–3275.
20. Matsuo, I.; Isomura, M.; Miyazaki, T.; Sakakibara, T.; Ajisaka, K. *Carbohydr. Res.* **1998**, *305*, 401–413.
21. Nicolaou, K. C.; Caulfield, T.; Kataoka, H.; Stylianides, N. A. *J. Am. Chem. Soc.* **1990**, *112*, 3693–3694.
22. (a) Konradsson, P.; Mootoo, D. R.; McDevitt, R. E.; Fraser-Reid, B. *J. Chem. Soc., Chem. Commun.* **1990**, 270–272;
(b) Veeneman, G. H.; van Leeuwen, S. H.; van Boom, J. H. *Tetrahedron Lett.* **1990**, *31*, 1331–1334.
23. Lönn, H. *J. Carbohydr. Chem.* **1987**, *6*, 301–306.
24. (a) Wormald, M. R.; Wooten, E. W.; Bazzo, R.; Edge, C. J.; Feinstein, A.; Rademacher, T. W.; Dwek, R. A. *Eur. J. Biochem.* **1991**, *198*, 131–139;
(b) Petrescu, A. J.; Butters, T. D.; Reinkensmeier, G.; Petrescu, S.; Platt, F. M.; Dwek, R. A.; Wormald, M. R. *EMBO J.* **1997**, *16*, 4302–4310;
(c) Woods, R. J.; Pathiaseril, A.; Wormald, M. R.; Edge, C. J.; Dwek, R. A. *Eur. J. Biochem.* **1998**, *258*, 372–386.