Carbohydrates

DOI: 10.1002/anie.201301613

Top-Down Chemoenzymatic Approach to a High-Mannose-Type Glycan Library: Synthesis of a Common Precursor and Its Enzymatic Trimming**

Akihiko Koizumi, Ichiro Matsuo, Maki Takatani, Akira Seko, Masakazu Hachisu, Yoichi Takeda, and Yukishige Ito**

Processing of asparagine (Asn)-linked (N-linked) glycans creates a variety of glycoforms which play important roles in a variety of intracellular events.[1] In the endoplasmic reticulum (ER) a combination of various enzymes, chaperones, lectins, and cargo receptors constitutes the glycoprotein quality control (GQC) system.[2]

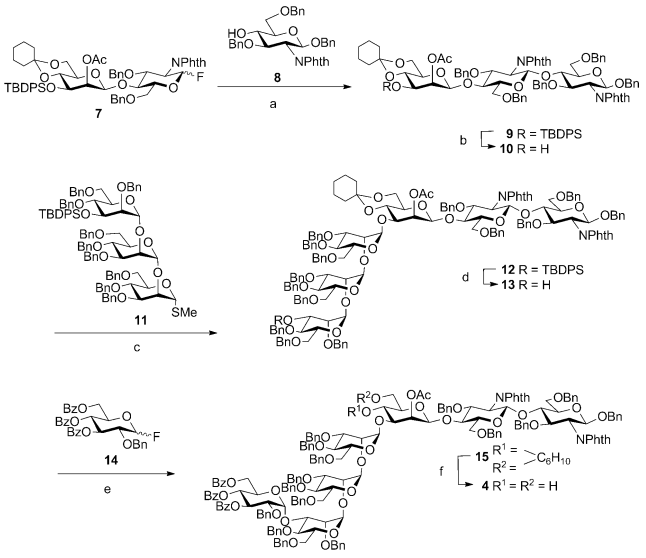
N-linked glycans are co-translationally introduced to nascent polypeptides initially as a triglucosylated tetradecasaccharide $Glc_3Man_9GlcNAc_2$ (G3M9)[3] by oligosaccharyltransferase (OST; Figure 1).[4] Subsequently, trimming by glucosidase I (G-I) and glucosidase II (G-II) produces the monoglucosylated glycoform $Glc_1Man_9GlcNAc_2$ (G1M9).[5] Further removal of the remaining Glc by G-II forms the nonglucosylated glycan $Man_9GlcNAc_2$ (M9). All the above-mentioned glycans are potentially susceptible to trimming by ER mannosidases,[6] which may generate glucosylated as well as nonglucosylated glycans with a variable number of Man residues (e.g. G1M8, G1M7, M8, M7, etc.).

Folding of glycoproteins is aided by a variety of chaperones in the ER.[7] Among them, calnexin (CNX) and calreticulin (CRT) occupy a privileged position as lectin chaperones, which are able to specifically recognize Glcα1→3Man-containing glycans, such as G1M9.[8]

In the ER, UDP-glucose:glycoprotein glucosyltransferase (UGGT) operates as the folding sensor, by which folding defective glycoproteins are re-glucosylated.[9] A combination of CNX/CRT, G-II, and UGGT constitutes a cycle (CNX/CRT cycle).[10] Simultaneously, misfolded glycoproteins are gradually demannosylated to $Man_8GlcNAc_2$ (M8), $Man_7GlcNAc_2$ (M7), and $Man_{5\text{-}6}GlcNAc_2$ (M5-6) by ER mannosidase and delivered to the cytosol for ER-associated degradation (ERAD).[11]

Our previous effort has established synthetic routes to most of the ER-related high-mannose-type glycans,[12] which have proven valuable in clarifying specificities of CRT,[8b,13] Fbs1,[14] UGGT,[15] and G-II.[16] To facilitate more comprehensive analysis of glycan functions in the GQC, we initiated the study to construct a high-mannose-type glycan library through top-down diversification of a common precursor.

Although our plan was hinted at by the biosynthetic pathway of N-linked glycans,[17] it is obviously unrealistic to reconstitute the biosynthetic pathway to generate the high-mannose glycan library. For instance, both G1M9 and M9 have multiple α1,2-mannosyl linkages, thus prohibiting controlled trimming of one of these Man residues (Figure 2a). To



Scheme 1. Synthesis of $Glc_1Man_4GlcNAc_2$ heptasaccharide acceptor. Reagents and conditions: a) $[Cp_2HfCl_2]$, AgOTf, 4 Å M.S., CH_2Cl_2, −10 °C, 3.5 h, 85 %. b) HF-pyridine/pyridine (1:5), 0 °C to RT, 21 h, 75 %. c) MeOTf in $ClCH_2CH_2Cl$, 4 Å M.S., CH_2Cl_2, −40 °C to RT, 12 h, 73 %. d) HF-pyridine, pyridine, 1 GPa, RT, 21 h, quant. e) $[Cp_2HfCl_2]$, AgOTf, 4 Å M.S., toluene/Et_2O (10:1), −40 to −10 °C, 21 h, 86 %. f) $TsOH{\cdot}H_2O$, MeCN, RT, 3 h, 73 %. Bz = benzoyl, $[Cp_2HfCl_2]$ = bis(cyclopentadienyl)hafnium dichloride, Phth = phthaloyl, M.S. = molecular sieves, Tf = trifluoromethanesulfonyl, Ts = 4-toluenesulfonyl.

[*] Dr. A. Koizumi, M. Takatani, Dr. A. Seko, Dr. M. Hachisu, Dr. Y. Takeda, Dr. Y. Ito
ERATO
Ito Glycotrilogy Project (Japan) Science and Technology Agency
2-1 Hirosawa, Wako, Saitama 351-0198 (Japan)
E-mail: yotakeda@riken.jp
yukito@riken.jp
Prof. I. Matsuo
Department of Chemistry and Chemical Biology, Gunma University
1-5-1 Tenjin-cho, Kiryu, Gunma 376-8515 (Japan)
Dr. Y. Ito
Synthetic Cellular Chemistry Laboratory, RIKEN
2-1 Hirosawa, Wako, Saitama 351-0198 (Japan)

[**] We thank Dr. Akihiro Ishiwata for his valuable advice as wells as Akemi Takahashi and Satoko Shirahata for their technical assistance. We are also grateful to Dr. Yoshio Sakaguchi and his staff of the Materials Characterization Team in RIKEN Advanced Support Division for elemental analyses.

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.201301613.

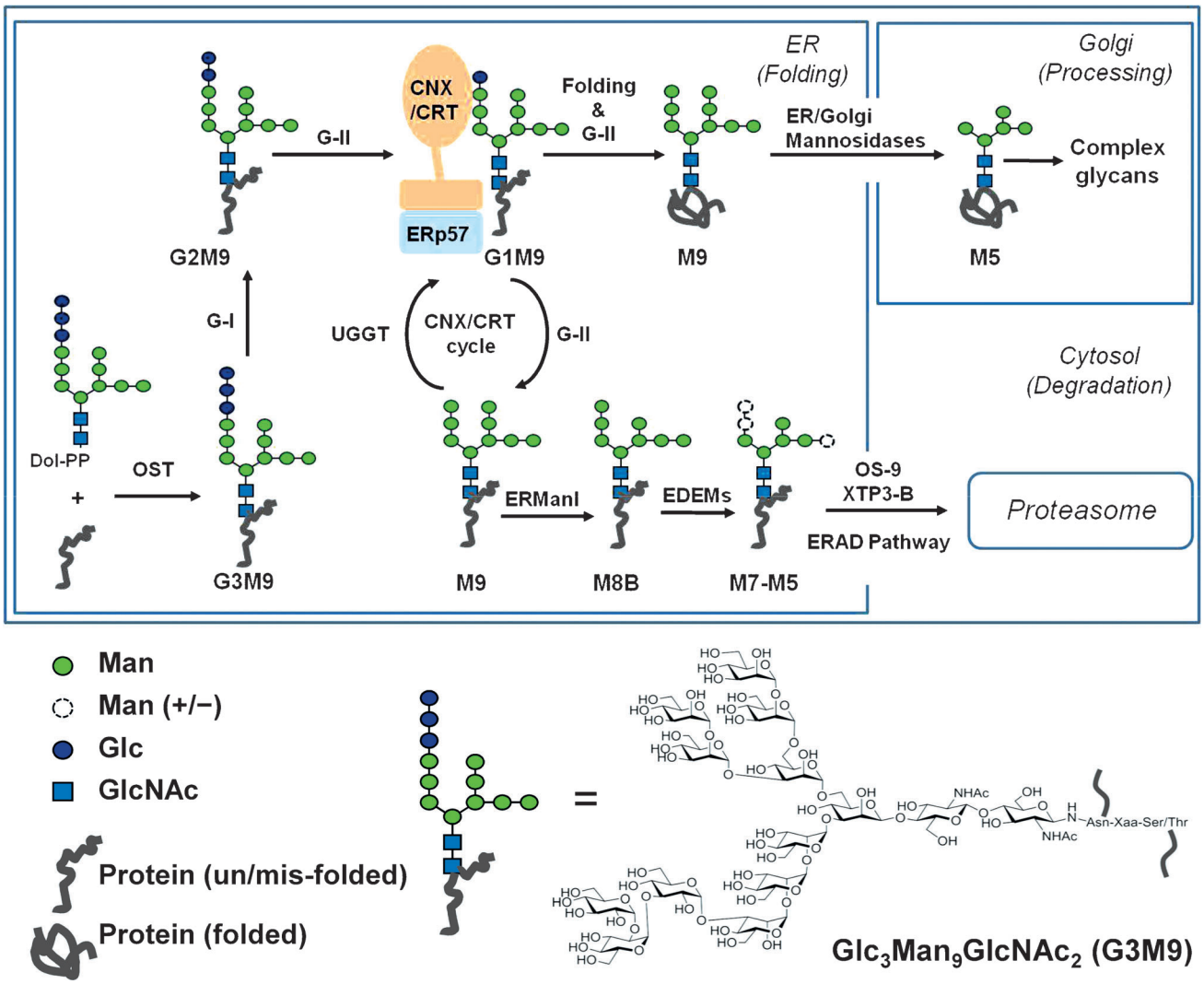


Figure 1. Glycoprotein quality control. OST = oligosaccharyltransferase, G-I = glucosidase I, G-II = glucosidase II, CNX = calnexin, CRT = calreticulin, ERManI = ER mannosidase I, UGGT = UDP-glucose:glycoprotein glucosyltransferase, EDEM = ER degradation-enhancing α-mannosidase-like protein, ERAD = ER-associated degradation.

overcome this problem, our strategy adopted the non-natural precursor **I**, each branch of which was capped by a distinct sugar residue (Figure 2b).[18] We anticipated that, if proper selection of the terminal sugar residues **A**, **B**, and **C** has been made, liberation of three arms can be executed independently, thus giving the second-generation precursors **I-A**, **I-B**, and **I-C**. Digestion with logically defined sets of glycosidases will give a variety of high-mannose-type glycans.

To pursue this scheme, we designed the non-natural tetradecasaccharide **1** as the precursor (Figure 3), in which A, B, and C branches were capped by α-Glc, β-Gal, and β-GlcNAc, respectively. We additionally planned to convert **1** into the BODIPY-labeled derivative **2** (BODIPY = boron-dipyrromethene). Our plan required the fully protected tetradecasaccharide **3**. As depicted in Figure 3, **3** was dissected into the three fragments **4**, **5**, and **6**.

To begin with, construction of the heptasaccharide acceptor **4**, which comprises the A arm and the core trisaccharide, was carried out as depicted in Scheme 1. Namely, the disaccharide (Manβ1-4GlcN) donor **7**,[12a] which was synthesized by intramolecular aglycon delivery,[19] was coupled with **8** to give the trisaccharide **9**, which was desilylated to provide **10**. Subsequent reaction with the thioglycoside **11**[12a] led to the hexasaccharide **12**, whose *tert*-butyldiphenylsilyl (TBDPS) group was removed[20] to give **13**. Its stereochemistry was confirmed by three signals, corresponding to α-mannosyl linkages, at $\delta > 5.0$ ppm ($\delta =$ 5.33 ppm, 5.25 ppm, 5.19 ppm). Subsequent glycosylation was conducted with the fluoride **14**.[12] The reaction was promoted by $[Cp_2HfCl_2]/AgOTf$[21] exclusively to give the desired isomer **15**, acid treatment of which gave the A-arm-containing heptasaccharide acceptor **4**.

For the construction of the C- and B-arm fragments, **6** and **5**, respectively, we opted to bifurcate the compound **16**.[22] Thus, treatment of **16** with $LiAlH_4/AlCl_3$[23] provided the 6-OH product **17** (Scheme 2), which was protected by a pentafluoropropionyl (PFP) group[24] to give **18**. The latter was used for glycosylation with the mannose-derived acceptor **19**,[25]

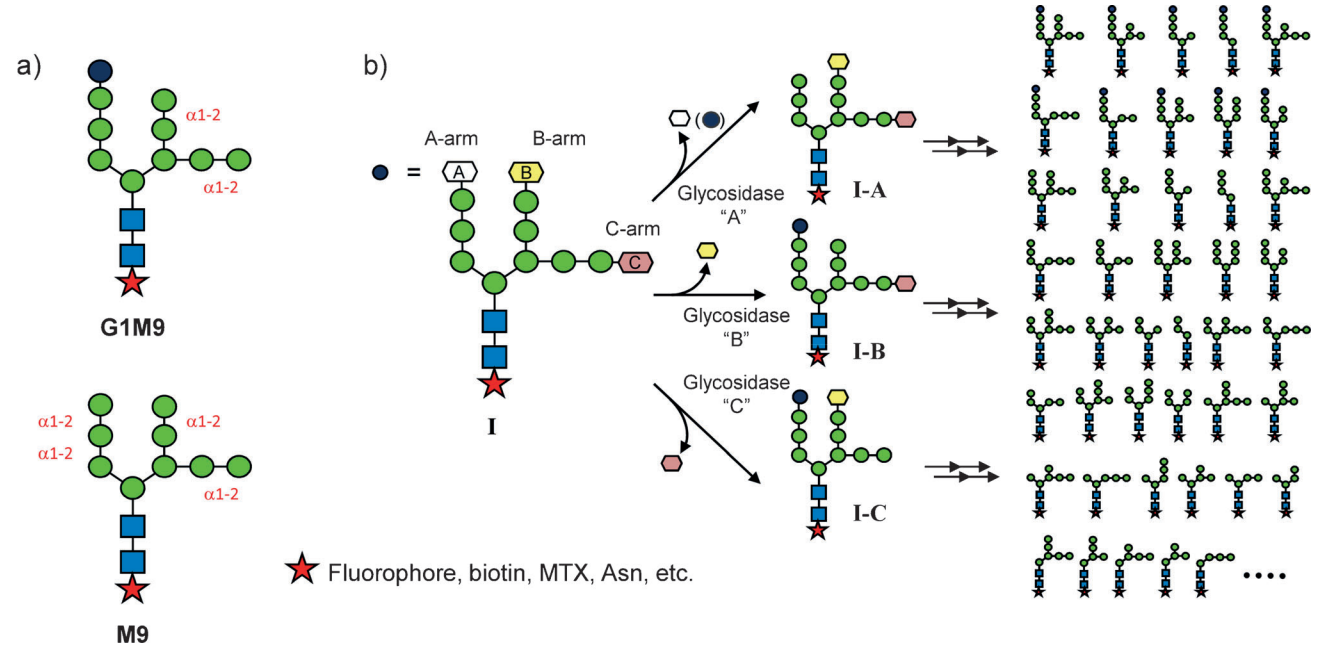


Figure 2. Overview of chemoenzymatic synthesis of high-mannose-type glycans. a) α1,2-Mannosidase-reactive sites of natural G1M9 and M9 glycans. b) Orthogonal liberation of A, B, and C arms of non-natural tetradecasaccharide for the construction of a high-mannose glycan library.

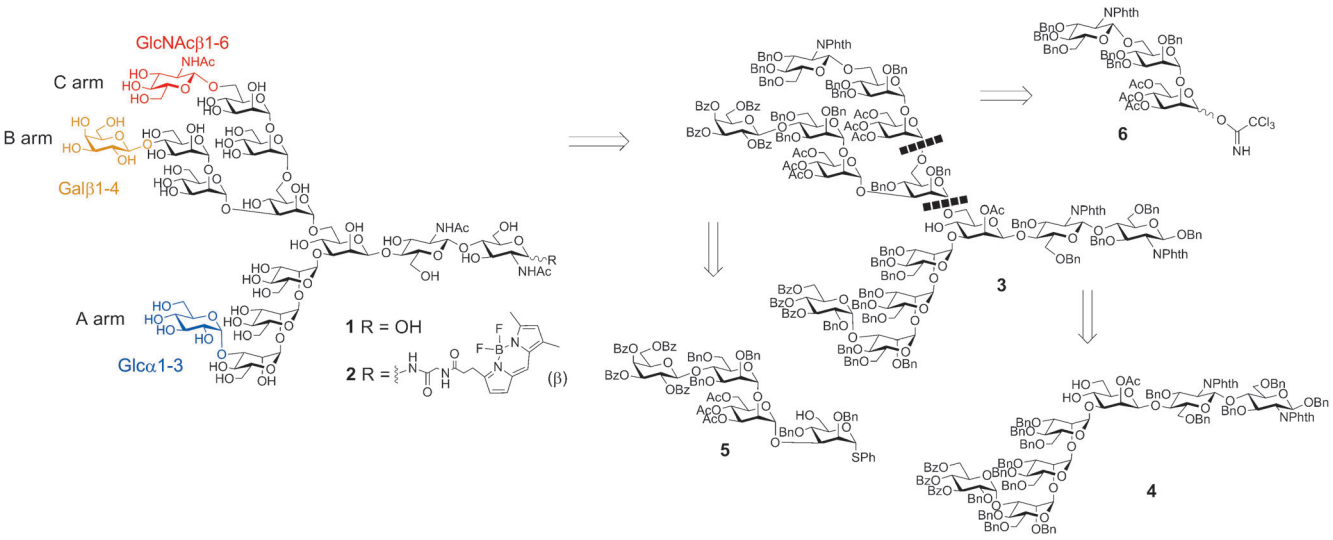


Figure 3. Retrosynthesis for the tetradecasaccharide **1** and BODIPY-labeled derivative **2**.

which gave the coupled product **20**. Removal of the PFP group was conducted under weakly basic conditions (pyridine/EtOH). The resultant **21** was subjected to coupling with the glucosaminyl donor **22**,[26] thus giving the trisaccharide **23**. Selective deacetylation gave **24**, which in turn was converted into the trichloroacetimidate **6**.

To construct the B arm, reductive opening of **16** was conducted by $NaBH_3CN$/HCl/1,4-dioxane[27] to provide **25**, which was again protected by PFP to give **26**. Glycosylation with **19** promoted by MeOTf[28] gave **27**, from which the PFP group was removed quantitatively. Thus the obtained compound **28** was glycosylated with the galactosyl chloride **29**[29] to give the trisaccharide **30**. The anomeric acetate **30** was converted into the trichloroacetimidate **32** via the hemiacetal **31**, which was coupled with the 6-*O*-chloroacetylated thioglycoside **33**,[30] thus giving the tetrasaccharide **34**. Its chloroacetyl group was removed[31] to give the tetrasaccharide acceptor **5**.

The compound **5** was then glycosylated with the C-arm donor **6** to give the heptasaccharide **35** (Scheme 3). Based on our previous experience,[12] the thioglycoside **35** was converted into the fluoride **36**. Coupling with the diol **4** gave **3**, which was fully deprotected to give **1**. Having the designed tetradecasaccharide in hand, fluorophoric modification was conducted to facilitate the screening of enzymatic reactions and analysis of glycan–protein interactions. The tetradeca-

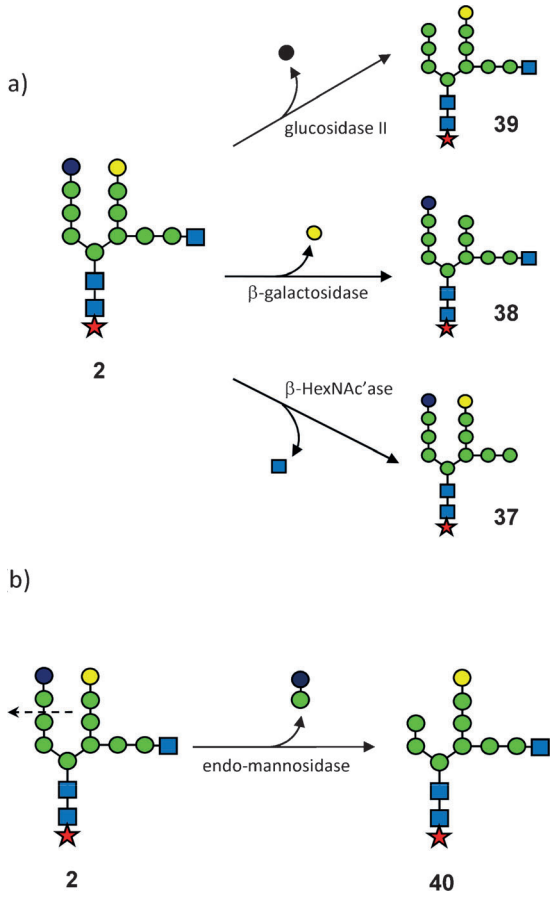
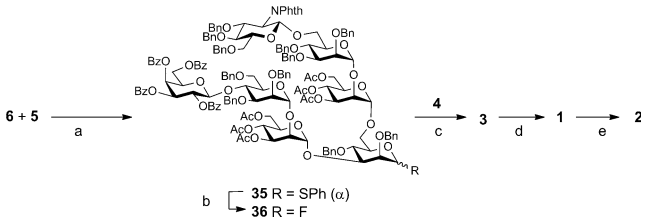
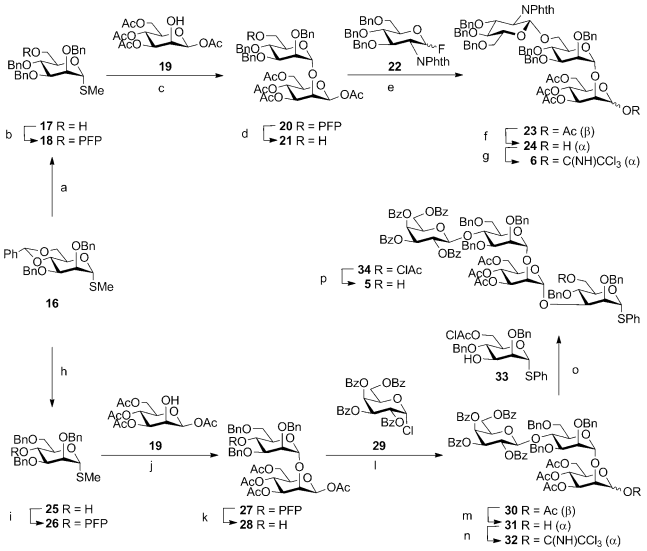


Figure 4. a) Enzymatic removal of GlcNAc, Gal, and Glc. b) Enzymatic digestion by Golgi *endo*-α-mannosidase.



Scheme 3. Synthesis of the tetradecasaccharide **1** and labeling with BODIPY. Reagents and conditions: a) TMSOTf, 4 Å M.S., CH_2Cl_2, −40 °C, overnight, 90 %. b) DAST, NBS, CH_2Cl_2, −40 to −20 °C, 3 h, 93 %. c) [Cp_2HfCl_2], AgOTf, 4 Å M.S., toluene, −40 °C to RT, overnight, 54 %. d) 1. ethylenediamine, *n*BuOH, 90 °C, 14 h; 2. Ac_2O, pyridine, RT, 19 h; 3. NaOMe, MeOH, 40 °C, 21 h; 4. $Pd(OH)_2/C$, H_2 (gas)/MeOH-H_2O, RT, 24 h, 59 %. e) 1. Sat. NH_4HCO_3 aq., 40 °C, overnight, 2. Fmoc-Gly-OPfp, pyridine, DMSO, 4 °C, 24 h; 3. piperidine, DMF, RT, 1 h; 4. BODIPY-FL-SE, DIPEA, DMF, RT, overnight, 55 % (4 steps). DAST = *N*,*N*-diethylaminosulfurtrifluoride, DMSO = dimethylsulfoxide, DIPEA = diisopropylethylamine, Fmoc = 9-fluorenylmethoxycarbonyl.

saccharide **1** was treated with aq. NH_4HCO_3,[32] thus giving the glycosylamine, which was reacted with Fmoc glycine pentafluorophenyl ester (Fmoc-Gly-OPfp) in DMSO at temperatures below freezing.[33] Removal of the Fmoc group and treatment with succinimide ester of BODIPY-FL gave the fluorescently-labeled tetradecasaccharide **2** (Scheme 3).



Scheme 2. Synthesis of the B-arm tetrasaccharide donor and the C-arm trisaccharide acceptor. Reagents and conditions: a) $LiAlH_4$, $AlCl_3$, Et_2O/CH_2Cl_2 (1:1), 50 °C, 1.5 h, 93 %. b) $(PFP)_2O$, pyridine, CH_2Cl_2, RT, 1.5 h, 93 %; c) DTBMP, MeOTf, 4 Å M.S., $ClCH_2CH_2Cl$, 50 °C, 15 h, 87 %. d) Pyridine, EtOH, RT, overnight, quant. e) [Cp_2HfCl_2], AgOTf, 4 Å M.S., CH_2Cl_2, −40 °C, 12 h, 84 %. f) NH_2NH_2·AcOH, DMF, RT, 2 h, 69 %; g) CCl_3CN, DBU, CH_2Cl_2, RT, 4 h, 84 %. h) $NaBH_3CN$, 4 M HCl in 1,4-dioxane, THF, 4 Å M.S., 0 °C, 9 h, 80 %. i) $(PFP)_2O$, pyridine, CH_2Cl_2, RT, 1.5 h, 96 %. j) DTBMP, MeOTf, 4 Å M.S., $ClCH_2CH_2Cl$, 50 °C, 15 h, 82 %. k) Pyridine, EtOH, 50 °C, overnight, quant. l) AgOTf in $ClCH_2CH_2Cl$, 4 Å M.S., toluene, 100 °C, 18 h, 71 %. m) NH_2NH_2·AcOH, DMF, RT, 1.5 h, 85 %. n) CCl_3CN, DBU, CH_2Cl_2, RT, 2 h, 87 %. o) TMSOTf, 4 Å M.S., CH_2Cl_2, −40 °C, overnight, 76 %. p) DABCO, EtOH/THF (2:1), RT, overnight, 91 %. DBU = 1,8-diazabicyclo[5.4.0]undec-7-ene, DMF = *N*,*N*-dimethylformamide, DTBMP = 2,6-di-*tert*-butyl-4-methylpyridine, THF = tetrahydrofuran, TMS = trimethylsilyl.

To remove the GlcNAc residue, Jack bean β-*N*-acetylhexosaminidase digestion was highly satisfactory, thus giving the tridecasaccharide **37** smoothly (Figure 4a). However, the β1,4-linked Gal residue was resistant to β-galactosidases derived from bovine liver, Jack bean, *Kluyveromyces lactis*, and *Escherichia coli*, presumably because of steric hindrance. Only *Aspergillus-oryzae*-derived enzyme had substantial activity to provide the B-arm-unblocked product **38**. Similarly, rat liver α-glucosidase II uniquely gave **39** by cleaving the Glc residue.

Although removal of the α-Glc from the A arm of **2** was straightforward, controlled removal of the terminal Man would be difficult. Consequently, digestion with Golgi *endo*-α-mannosidase was conducted, and cleanly gave **40** (Figure 4b). Importantly, the presence of terminal α-Glc conferred susceptibility to endomannosidase.[34]

To demonstrate the feasibility of our strategy, we attempted systematic preparation of mono- as well as non-glucosylated M8 glycans (Figure 5), because of their important roles in GQC.[2] Production of M8A was achieved by a three-step enzymatic trimming with Jack bean β-*N*-acetylhexosaminidase, Golgi *endo*-α-mannosidase, and *A. oryzae* β-galactosidase. HPLC profiles for each step are shown in Figure S1 of the Supporting Information. G1M8(B) was given

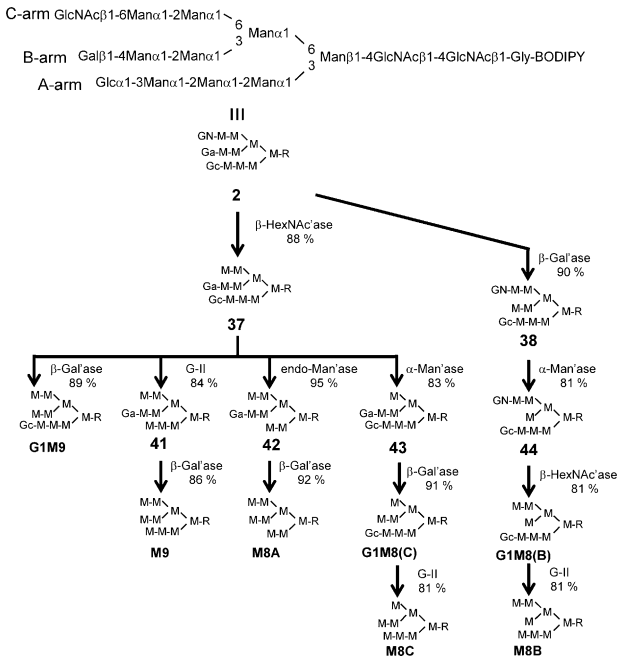


Figure 5. Preparation of G1M9, G1M8(B), G1M8(C), M9, M8A, M8B, and M8C from the tetradecasaccharide. Golgi *endo*-α-mannosidase (*endo*-Man′ase), Jack bean β-*N*-acetylhexosaminidase (β-HexNAc′ase), *Aspergillus oryzae* β-galactosidase (β-Gal′ase), *Aspergillus saitoi* α1,2-mannosidase (α-Man′ase), and rat liver α-glucosidase-II (G-II) were used. GN = *N*-acetylglucosamine, M = mannose, Ga = galactose, Gc = glucose.

by *A. oryzae* β-galactosidase digestion, followed by sequential treatment with *Aspergillus saitoi* α1,2-mannosidase and Jack bean β-*N*-acetylhexosaminidase. Similarly, its regioisomer, G1M8(C), was obtained by sequential digestion with β-*N*-acetylhexosaminidase, α1,2-mannosidase, and β-galactosidase. Glucosidase II treatment of these undecasaccharides smoothly gave the nonglucosylated congeners M8B and M8C. In support of their structures, M8A, M8B, and M8C gave the same molecular mass (MW = 2074.5), but exhibited different retention times (Figure S2). Further evidence of their structures was obtained by *A. saitoi* α1,2-mannosidase digestion, which gave identical Man5 derivative (MW = 1589.0) from all three isomers (Figure S3). Similarly, after the α1,2-mannosidase digestion, G1M8(B), G1M8(C), and G1M9 gave the same G1M7 derivative (MW = 2074.5).[35] Although these enzymatic digestions were performed on the pmol mL^{-1} scale, this method can be easily extended to the μmol mL^{-1} scale, which is sufficient for various bioassays.

In summary, we achieved efficient synthesis of the tetradecasaccharide **1**, which was shown to be appropriate as the common precursor of top-down, chemoenzymatic synthesis of high-mannose glycans. This method will provide a powerful tool in studying biological events related to high-mannose-type glycans.

Received: February 25, 2013
Revised: May 1, 2013
Published online: June 6, 2013

Keywords: carbohydrates · enzymes · glycopeptides · protein folding · synthesis design

[1] a) D. N. Hebert, S. C. Garman, M. Molinari, *Trends Cell Biol.* **2005**, *15*, 364 – 370; b) A. J. Parodi, *Biochem. J.* **2000**, *348*, 1 – 13.
[2] a) D. N. Hebert, M. Molinari, *Physiol. Rev.* **2007**, *87*, 1377 – 1408; b) R. Benyair, E. Ron, G. Z. Lederkremer, *Int. Rev. Cell Mol. Biol.* **2011**, *292*, 197 – 280.
[3] a) S. J. Turco, B. Stetson, P. W. Robbins, *Proc. Natl. Acad. Sci. USA* **1977**, *74*, 4411 – 4414; b) S. J. Turco, P. W. Robbins, *J. Biol. Chem.* **1979**, *254*, 4560 – 4567.
[4] a) E. Bause, *Biochem. J.* **1983**, *209*, 331 – 336; b) P. Whitley, I. M. Nilsson, G. von Heijne, *J. Biol. Chem.* **1996**, *271*, 6241 – 6244; c) A. X. Yan, W. J. Lennarz, *J. Biol. Chem.* **2005**, *280*, 3121 – 3124.
[5] E. S. Trombetta, *Glycobiology* **2003**, *13*, 77R – 91R.
[6] S. W. Mast, K. W. Moremen, *Methods Enzymol.* **2006**, *415*, 31 – 46.
[7] a) P. Maattanen, K. Gehring, J. J. M. Bergeron, D. Y. Thomas, *Semin. Cell Dev. Biol.* **2010**, *21*, 500 – 511; b) L. A. Rutkevich, D. B. Williams, *Curr. Opin. Cell Biol.* **2011**, *23*, 157 – 166.
[8] a) A. R. Patil, C. J. Thomas, A. Surolia, *J. Biol. Chem.* **2000**, *275*, 24348 – 24356; b) A. Tatami, Y. S. Hon, I. Matsuo, M. Takatani, H. Koshino, Y. Ito, *Biochem. Biophys. Res. Commun.* **2007**, *364*, 332 – 337; c) F. E. Ware, A. Vassilakos, P. A. Peterson, M. R. Jackson, M. A. Lehrman, D. B. Williams, *J. Biol. Chem.* **1995**, *270*, 4697 – 4704.
[9] a) J. J. Caramelo, O. A. Castro, L. G. Alonso, G. De Prat-Gay, A. J. Parodi, *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 86 – 91; b) J. J. Caramelo, O. A. Castro, G. de Prat-Gay, A. J. Parodi, *J. Biol. Chem.* **2004**, *279*, 46280 – 46285; c) S. C. Taylor, A. D. Ferguson, J. J. Bergeron, D. Y. Thomas, *Nat. Struct. Mol. Biol.* **2004**, *11*, 128 – 134.
[10] a) J. J. Caramelo, A. J. Parodi, *J. Biol. Chem.* **2008**, *283*, 10221 – 10225; b) L. Ellgaard, E. M. Frickel, *Cell Biochem. Biophys.* **2003**, *39*, 223 – 247.
[11] J. C. Christianson, T. A. Shaler, R. E. Tyler, R. R. Kopito, *Nat. Cell Biol.* **2008**, *10*, 272 – 282.
[12] a) I. Matsuo, M. Wada, S. Manabe, Y. Yamaguchi, K. Otake, K. Kato, Y. Ito, *J. Am. Chem. Soc.* **2003**, *125*, 3402 – 3403; b) I. Matsuo, K. Totani, A. Tatami, Y. Ito, *Tetrahedron* **2006**, *62*, 8262 – 8277.
[13] M. A. Arai, I. Matsuo, S. Hagihara, K. Totani, J. Maruyama, K. Kitamoto, Y. Ito, *ChemBioChem* **2005**, *6*, 2281 – 2289.
[14] S. Hagihara, K. Totani, I. Matsuo, Y. Ito, *J. Med. Chem.* **2005**, *48*, 3126 – 3129.
[15] a) K. Totani, Y. Ihara, K. Matsuo, H. Koshino, Y. Ito, *Angew. Chem.* **2005**, *117*, 8164 – 8168; *Angew. Chem. Int. Ed.* **2005**, *44*, 7950 – 7954; b) K. Totani, Y. Ihara, T. Tsujimoto, I. Matsuo, Y. Ito, *Biochemistry* **2009**, *48*, 2933 – 2940; c) A. Miyagawa, K. Totani, I. Matsuo, Y. Ito, *Biochem. Biophys. Res. Commun.* **2010**, *403*, 322 – 328.
[16] a) K. Totani, Y. Ihara, I. Matsuo, Y. Ito, *J. Biol. Chem.* **2006**, *281*, 31502 – 31508; b) K. Totani, Y. Ihara, I. Matsuo, Y. Ito, *J. Am. Chem. Soc.* **2008**, *130*, 2101 – 2107; c) Y. Takeda, K. Totani, I. Matsuo, Y. Ito, *Bioorg. Med. Chem. Lett.* **2010**, *20*, 5357 – 5359.
[17] a) A. Helenius, M. Aebi, *Annu. Rev. Biochem.* **2004**, *73*, 1019 – 1049; b) L. W. Ruddock, M. Molinari, *J. Cell Sci.* **2006**, *119*, 4373 – 4380.
[18] Kajihara et al. developed an elegant chemoenzymatic approach toward complex-type glycans, in which an egg-yolk-derived glycopeptide was employed as a common precursor:Y. Kajihara, Y. Suzuki, N. Yamamoto, K. Sasaki, T. Sakakibara, L. R. Juneja, *Chem. Eur. J.* **2004**, *10*, 971 – 985.
[19] a) F. Barresi, O. Hindsgaul, *J. Am. Chem. Soc.* **1991**, *113*, 9376 – 9377; b) Y. Ito, Y. Ohnishi, T. Ogawa, Y. Nakahara, *Synlett* **1998**,

1102 – 1104; c) A. Ishiwata, Y. J. Lee, Y. Ito, *Org. Biomol. Chem.* **2010**, *8*, 3596 – 3608; d) A. Ishiwata, A. Sakurai, Y. Nishimiya, S. Tsuda, Y. Ito, *J. Am. Chem. Soc.* **2011**, *133*, 19524 – 19535.
[20] I. Matsuo, M. Wada, Y. Ito, *Tetrahedron Lett.* **2002**, *43*, 3273 – 3275.
[21] K. Suzuki, H. Maeta, T. Matsumoto, G. Tsuchihashi, *Tetrahedron Lett.* **1988**, *29*, 3571 – 3574.
[22] A. Ishiwata, A. Sakurai, K. Durr, Y. Ito, *Bioorg. Med. Chem.* **2010**, *18*, 3687 – 3695.
[23] A. Lipták, I. Jodal, P. Nanasi, *Carbohydr. Res.* **1975**, *44*, 1 – 11.
[24] M. Takatani, I. Matsuo, Y. Ito, *Carbohydr. Res.* **2003**, *338*, 1073 – 1081.
[25] J. O. Deferrari, E. G. Gros, I. O. Mastronardi, *Carbohydr. Res.* **1967**, *4*, 432 – 434.
[26] Y. Ohnishi, H. Ando, T. Kawai, Y. Nakahara, Y. Ito, *Carbohydr. Res.* **2000**, *328*, 263 – 276.
[27] P. J. Garegg, H. Hultberg, S. Wallin, *Carbohydr. Res.* **1982**, *108*, 97 – 101.
[28] H. Lonn, *Carbohydr. Res.* **1985**, *139*, 105 – 113.
[29] C. W. Chang, S. S. Chang, C. S. Chao, K. K. T. Mong, *Tetrahedron Lett.* **2009**, *50*, 4536 – 4540.
[30] I. Matsuo, T. Miyazaki, M. Isomura, T. Sakakibara, K. Ajisaka, *J. Carbohydr. Chem.* **1998**, *17*, 1249 – 1258.
[31] D. J. Lefeber, J. P. Kamerling, J. F. G. Vliegenthart, *Org. Lett.* **2000**, *2*, 701 – 703.
[32] L. M. Likhosherstov, O. S. Novikova, V. A. Derevitskaja, N. K. Kochetkov, *Carbohydr. Res.* **1986**, *146*, C1 – C5.
[33] a) R. E. Pincock, T. E. Kiovsky, *J. Am. Chem. Soc.* **1966**, *88*, 51 – 55; b) N. H. Grant, H. E. Alburn, *Science* **1965**, *150*, 1589 – 1590; c) B. N. Naidu, W. Y. Li, M. E. Sorenson, T. P. Connolly, J. A. Wichtowski, Y. H. Zhang, O. K. Kim, J. D. Matiskella, K. S. Lam, J. J. Bronson, Y. Ueda, *Tetrahedron Lett.* **2004**, *45*, 1059 – 1063; d) M. Takatani, J. Nakano, M. A. Arai, A. Ishiwata, H. Ohta, Y. Ito, *Tetrahedron Lett.* **2004**, *45*, 3929 – 3932.
[34] W. A. Lubas, R. G. Spiro, *J. Biol. Chem.* **1988**, *263*, 3990 – 3998.
[35] HPLC profiles and MALDI TOF-MS data of all glycans are provided in the Supporting Information (Table S1).

