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Journal

Chemical Communications, 51(58)

ISSN

1359-7345

Authors

Zhang, Yan
Meng, Caicai
Jin, Lan
et al.

Publication Date

2015-07-25

DOI

10.1039/c5cc02913a

Peer reviewed



Published in final edited form as:

Chem Commun (Camb). 2015 July 25; 51(58): 11654–11657. doi:10.1039/c5cc02913a.

Chemoenzymatic synthesis of α -dystroglycan core M1 O-mannose glycans

Yan Zhang^a, Caicai Meng^a, Lan Jin^a, Xi Chen^b, Fengshan Wang^{a,c}, and Hongzhi Cao^a

Xi Chen: xiichen@ucdavis.edu; Fengshan Wang: fswang@sdu.edu.cn; Hongzhi Cao: hzcao@sdu.edu.cn

^aNational Glycoengineering Research Center, School of Pharmaceutical Science, Shandong University, Jinan 250012, China

^bDepartment of Chemistry, University of California, One Shields Avenue, Davis, California 95616, USA

^cKey Laboratory of Chemical Biology(Ministry of Education), Shandong University, Jinan 250012, China

Abstract

The diversity-oriented chemoenzymatic synthesis of α -dystroglycan (α -DG) core M1 O-mannose glycans has been achieved via a three-step sequential one-pot multienzyme (OPME) glycosylation of a chemically prepared disaccharyl serine intermediate. The high flexibility and efficiency of this chemoenzymatic strategy was demonstrated for the synthesis of three more complex core M1 O-mannose glycans for the first time along with three previously reported core M1 structures.

α -Dystroglycan (α -DG), a heavily glycosylated protein found in muscle and brain tissue, is a key component of the dystroglycan-glycoprotein complex that links the extracellular matrix to the intracellular cytoskeleton.^{1–4} In addition to conventional N-glycans and O-GalNAc glycans, the “uncommon” O-mannose glycans abundant on the mucin domain of α -DG play essential roles in muscle structure and function. Defects in O-mannosylation can cause several congenital muscular dystrophies (CMDs) and promote metastasis of many types of cancers.^{1,2,5–7} Moreover, α -DG is a host cell surface receptor for a number of arenaviruses, including lymphocytic choriomeningitis virus (LCMV) and Lassa fever virus (LFV).^{1,2,6}

So far, more than 50 mammalian O-mannosylated proteins have been identified, over 20 O-mannose glycan structures have been well characterized, and it is estimated that the O-mannose glycans account for up to 30% of all O-glycans in mammalian brain tissue.¹ However, α -DG is the only O-mannosylated protein which has been intensively studied. The core M1 structures are account for at least 50% of the total O-mannose glycans in brain, and the sialyl tetrasaccharide structure (**III** in Figure 1) has been identified as the most abundant form of core M1 glycans.¹

Correspondence to: Fengshan Wang, fswang@sdu.edu.cn; Hongzhi Cao, hzcao@sdu.edu.cn.

[†]Electronic Supplementary Information (ESI) available: Experimental details, characterization data, and copies of NMR (¹H and ¹³C) spectra of all key intermediates and final products. See DOI: 10.1039/b000000x/

Although the biological significance of O-mannose glycans has been well documented, the biosynthetic pathway and the precise function of this diverse glycan library have not yet been fully understood.^{1–6} Therefore, the synthesis of structurally well-defined O-mannose glycans in sufficient amount will not only provide carbohydrate standards for the identification of these glycans from natural sources, but also provide the probes for the elucidation of their biosynthetic pathway and structure-activity relationship (SAR) studies.

Despite the tremendous advances made in the chemical glycosylation processes in the past few decades, the stereoselective formation of the sialic acid linkage is still challenging.^{7–11} The overall synthetic strategy of core M1 O-mannosyl amino acids was further complicated by the lability of sialic acid and fucose residues^{12,13}, and the racemization-prone amino acid moiety^{14, 15}. Therefore, despite the O-mannose glycan structure **III** (Figure 1) was first identified by Endo and co-workers in 1997¹⁶, and over 20 structurally distinct O-mannose glycans have been reported thereafter, only four of them have been synthesized by 5 research groups around the world.^{17–22} The previous chemical or chemoenzymatic synthesis mainly focused on the synthesis of core M1 tetrasaccharide structure **III**^{17–21} or the phosphorylated core M3 trisaccharide²². However, the Neu5Gc-, Le^x- and sLe^x-containing more complex core M1 structures **IV**, **VI**, and **VII** (Figure 1) have not been synthesized in any forms. Therefore, there is an urgent need to develop a diversity-oriented synthesis to access these structurally distinct complex O-mannose glycans. We have successfully achieved this using a sequential three-step one-pot multienzyme (OPME) glycosylation process.

The retrosynthetic plan for the diversity-oriented chemoenzymatic synthesis of core M1 O-mannose glycans **2–7** is depicted in Scheme 1. The chemical synthesized disaccharide O-mannosyl serine **2** was chosen as the key intermediate and starting point for our sequential OPME synthesis of core M1 O-mannose glycans **3–7** (Scheme 1). The disaccharide O-mannosyl serine **2**, which in turn could be prepared from disaccharide thioglycoside **1** by using strategy of late stage introduction of amino acid. Subsequently, the disaccharyl serine **2** can serve as the substrate for a sequential OPME glycosylation process to afford the core M1 O-mannose glycans **3–7** using OPME β 1–4 galactosylation (OPME 1)^{23,24}, α 2–3-sialylation(OPME 2)²⁵ and α 1–3-fucosylation (OPME 3)^{24,26} reactions (Scheme 1).

The chemical synthesis of disaccharide O-mannosyl serine **2** was commenced with preparation of bifunctional thiomannoside **10** (Scheme 2). The readily available thiomannoside **8**²⁷ was transformed into the corresponding 2,6-diol **9** in one step in 89% yield using Ley's diketone protection protocol^{28,29}. The C6–OH of **9** was selectively protected as a *tert*-butyldimethylsilyl (TBDMS) ether to give mannoside **10** in 93% yield in the presence of TBDMSCl and imidazole at room temperature. The resulting thiomannoside **10** was then glycosylated with a known glucosamine trichloroacetimidate donor **11**³⁰ in the presence of trimethylsilyl trifluoromethanesulfonate (TMSOTf) to give disaccharide **12** in 95% yield.

The protecting group manipulation of **12** to give disaccharide donor **1** was executed prior to the introduction of the amino acid moiety in a four-step procedure. Briefly, the TBDMS silyl in **12** group was removed using HF-pyridine to furnish disaccharide **13** in 94% yield. The

C3,C4-diketone deprotection was achieved by treating **13** with trifluoroacetic acid/water (9:1, v/v) for 30 minutes to provide the disaccharide **14** in 91% yield. Finally, the deacetylation including the removal of the *N*-trifluoroacetyl group was performed under basic condition, and the resulting disaccharide **15** was peracetylated to afford thioglycoside **1** in 68% yield for two steps.

Coupling of disaccharide donor **1** with serine derivative **16**³¹ in the presence of *N*-iodocoussinimide (NIS) and catalytic amount of silver trifluoromethanesulfonate (AgOTf) produced the α -linked disaccharyl serine **17** as the only product in 94% yield. The nascent glycosidic linkage was determined as α since the coupling constant ($^1J_{\text{CH}} = 173.4$ Hz) between the anomeric carbon and hydrogen atom was in the range of α -manno configurate O-glycoside.^{32,33} The fully protected disaccharyl serine **17** was converted to the Fmoc-protected disaccharyl serine **2** in 72% yield for two steps including trifluoroacetic acid mediated deprotection of *tert*-butyl ester and deacetylation under Zemplén condition (Scheme 2).

With the chemically synthesized disaccharyl serine **2** in hand, two OPME glycosylation (OPME 1, OPME β 1–4-galactosylation and OPME 2, OPME α 2–3-sialylation) were investigated for the biomimetic stepwise chain elongation to afford trisaccharide O-mannose glycan **3**, tetrasaccharide O-mannose glycans **4** and **5**, respectively (Scheme 3).

The OPME β 1–4-galactosylation of disaccharyl serine acceptor **2** was performed on preparative scales (100 mg) in 100 mM Tris-HCl buffer (pH 7.5) in the presence of UDP-Glc, MgCl₂ (20 mmol), an *Escherichia coli* UDP-galactose-4-epimerase (EcGalE)³⁴ and a *Neisseria meningitidis* β 1–4GalT (NmLgtB)²³ at 37 °C for 16 hours. In this one-pot two-enzyme system, the commercially available UDP-Glc was epimerized by EcGalE to afford UDP-Gal which was then used by NmLgtB to form the required β 1–4-linkage. To our delight, the disaccharyl serine **2** can be well tolerated by NmLgtB to afford the desired trisaccharyl serine **3** in 89% yield. Previously, Paulson and co-workers found that the UDP-Glc also can be used by NmLgtB to give the glucosylated disaccharide product in 13% yield in the absence of GalE, while no glucosylated by-product was detected when the NmLgtB-catalyzed reaction was coupled with GalE.³⁵ The structure of trisaccharide **3** was confirmed by extensive 1D and 2D NMR studies (SI), as the chemical shifts of H2 (3.53 ppm) and H4 (3.89 ppm) of the galactose residue were consistent with previous report [H2 (3.55 ppm) and H4 (3.92 ppm)]¹⁸, in contrast, the chemical shifts of H2 and H4 of glucose residue were usually in the range of 3.31 ppm and 3.41 ppm, respectively³⁶.

Having successfully demonstrated the OPME β 1–4-galactosylation using disaccharyl serine **2** as acceptor, we next anticipated that the OPME α 2–3-sialylation²⁵ might also be capable of using this galactosyl trisaccharide **3** as substrate. As shown in Scheme 3, the one-pot three-enzyme sialylation of trisaccharide **3** (150 mg) was performed in 100 mM Tris-HCl buffer (pH 8.5) in the presence of *N*-acetyl-mannosamine (ManNAc, **19**), sodium pyruvate, cytidine 5'-triphosphate (CTP), MgCl₂ (20 mmol), a *Pasteurella multocida* sialic acid aldolase (Pm aldolase)³⁷, a *Neisseria meningitidis* CMP-sialic acid synthetase (NmCSS)³⁸ and a *Pasteurella multocida* multifunctional α 2–3-sialyltransferase (PmST1)²⁵ at 37 °C for 30 min. The Pm aldolase is responsible for converting ManNAc to *N*-acetylneuraminic acid

(Neu5Ac) in the presence of sodium pyruvate. The Neu5Ac was then converted to CMP-Neu5Ac (**21**) catalyzed by NmCSS in the presence of cytidine 5'-triphosphate (CTP), and the *in situ* generated CMP-Neu5Ac (**21**) was utilized by PmST1 to form the desired α 2–3-linked sialoside **4** in 92% yield (Scheme 3). Following the same approach, the *N*-glycolylneuraminic acid (Neu5Gc)-containing tetrasaccharyl serine **5** was also prepared in one-pot in 95% yield using *N*-glycolyl-mannosamine (ManNGc, **20**) as a sialic acid precursor (Scheme 3). It is noteworthy that the Neu5Gc-containing O-mannose tetrasaccharide **5** was synthesized for the first time.

For the synthesis of fucosyl O-mannose glycans **6** and **7**, a one-pot two-enzyme system (OPME 3)^{24,26} was employed to stereo- and regioselective introducing α 1–3-linked fucose to the trisaccharide acceptor **3** and tetrasaccharide acceptor **4**, respectively. As shown in Scheme 4, in this system, L-fucose was activated by the bifunctional L-fucokinase/GDP-fucose pyrophosphorylase (FKP) from *Bacteroides fragilis*³⁹ in the presence of adenosine 5'-triphosphate (ATP) and guanosine 5'-triphosphate (GTP) to form GDP-Fuc via a fucose-1-phosphate (Fuc-1-P) intermediate. The *in situ* generated GDP-Fuc was then taken by a recombinant N-His₆-tagged C-terminal truncated *Helicobacter pylori* α 1–3-fucosyltransferase (Hp α 1–3FT 66)^{26,40} to form the desired Le^x-containing O-mannose glycan **6** (200 mg) in 85% yield after purification. The synthesis of sialyl Le^x-containing O-mannose glycan **7** from tetrasaccharide **4** was performed following the same procedure as described above for **6** (Scheme 4). The pentasaccharide **7** (35 mg) was obtained in 74% yield. It is noteworthy that both Le^x- and sialyl Le^x-containing O-mannose glycans **6** and **7** were synthesized for the first time.

In summary, we have developed a highly efficient diversity-oriented chemoenzymatic strategy for the rapid assembly of core M1 O-mannose glycans **2–7** as their Fmoc-Ser-linked derivatives. The success of this strategy relies on the stepwise elongation of a chemically produced disaccharide intermediate **2** using three highly efficient OPME glycosylation reactions. The concise chemical synthesis of disaccharide **2** by means of Ley's diketone protection group and introducing the amino acid moiety at late stage provide a novel entry for gram-scale production of core M1 disaccharides without the aid of mammalian enzymes. It is worth to notice that the Neu5Gc-, Le^x- and sLe^x-containing O-mannose glycans **5–7** were synthesized for the first time.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

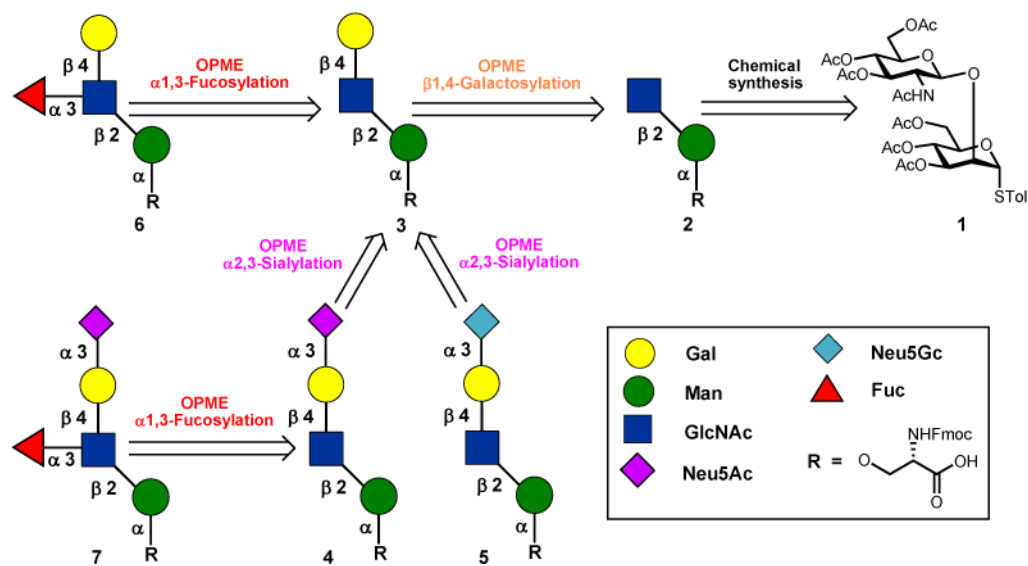
Acknowledgments

This work was financially supported by the Major State Basic Research Development Program of China (973 Program, No. 2012CB822102 to H. C. and F. W.), the National High Technology Research and Development Program of China (2012AA021504 to H.C.), National Natural Science Foundation of China (Grant Nos. 21172135, 21372145 to H. C.), Development Fund for Collaborative Innovation Center of Glycoscience of Shandong University and NIH grant R01HD065122 (to X. C.).

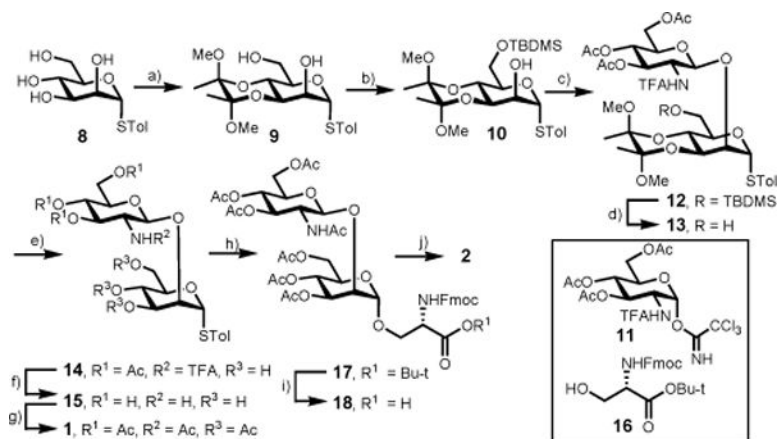
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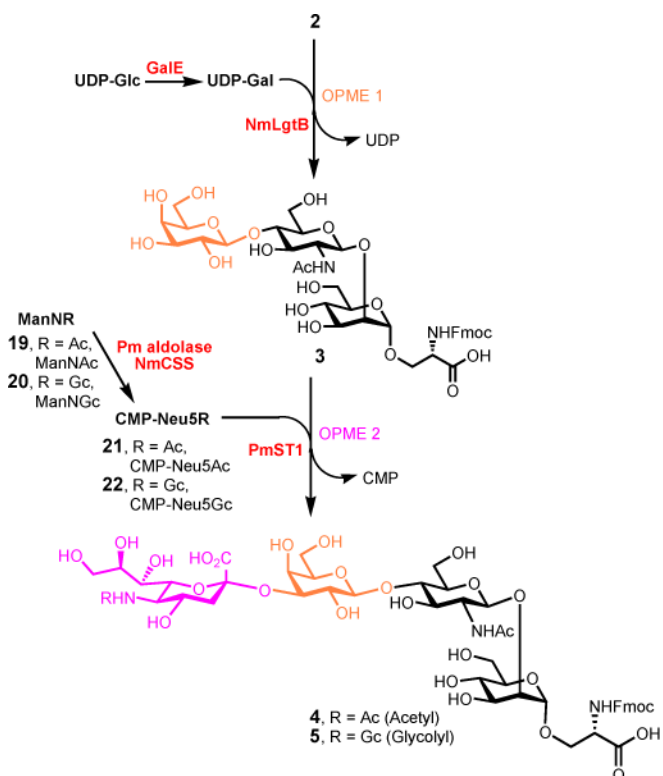
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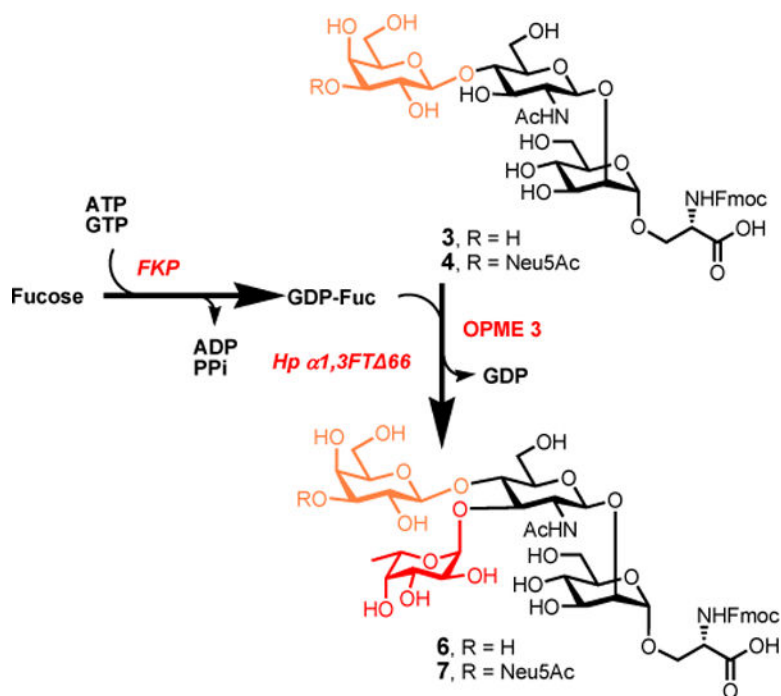
Scheme 1.
Retrosynthetic plan for core M1 α -DG glycans 2–7

**Scheme 2.**

Chemical synthesis of disaccharyl serine **2**. *Reagents and conditions*: a) 2,3-butanedione (1.1 equiv), trimethyl orthoformate (3.3 equiv), (\pm) camphorsulfonic acid (CSA, 0.11 equiv), 110 °C, 12 h, 89%; b) TBDMSCl (1.5 equiv), imidazole (3.0 equiv), DMF, rt, overnight, 93%; c) **11** (1.5 equiv) TMSOTf (0.2 equiv), 4 Å MS, CH₂Cl₂, -30 °C, 30 min, 95%; d) pyridine-HF, rt, overnight, 94%; e) TFA/H₂O (9:1, v/v), 30 min, 91%; f) MeOH/NaOMe pH 11, 20 min, then 1 M LiOH, 1 h; g) Ac₂O, pyridine, rt, overnight, 68 % for two steps; h) **16** (1.5 equiv) NIS (1.5 equiv), AgOTf (0.2 equiv), CH₂Cl₂, 4 Å MS, -25 °C, 1 h, 94%; i) TFA/H₂O (10:1, v/v), rt, 30 min; j) MeOH/NaOMe pH 8.5, rt, 40 min, 72% for two steps.

**Scheme 3.**

Sequential OPME synthesis of trisaccharyl serine **3**, tetrasaccharyl serine **4** and **5**. *Reagents and conditions:* OPME 1, UDP-Glc (1.3 equiv), MgCl_2 (20 mmol), Tris-HCl buffer (100 mmol, pH 7.5), EcGalE, NmLgtB, 37 °C, 16 h, 89% for **3** in one-pot; OPME 2, ManNAc (2.0 equiv, for **4**), ManNGc (2.0 equiv, for **5**), pyruvate (4.0 equiv), CTP (2.0 equiv), MgCl_2 (20 mmol), Tris-HCl buffer (100 mmol, pH 8.5), Pm aldolase, NmCSS, PmST1, 37 °C, 30 min, 92% for **4**, 95% for **5**.



Scheme 4.

Enzymatic synthesis of fucosyl *O*-mannose glycans **6** and **7** using OPME fucosylation.

Reagents and conditions: L-fucose (1.3 equiv), ATP (1.3 equiv), GTP (1.3 equiv), MnCl₂ (20 mM), Tris-HCl buffer (100 mM, pH 7.5), FKP, Hp α 1-3FT Δ 66, 37 °C, 12 h, 85% for **6**, 74% for **7**.