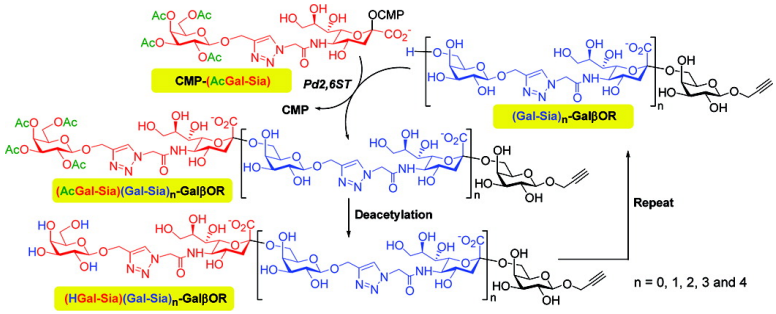
Communication

Chemoenzymatic Synthesis of Size-Defined Polysaccharides by Sialyltransferase-Catalyzed Block Transfer of Oligosaccharides

Saddam Muthana, Hai Yu, Shengshu Huang, and Xi Chen

J. Am. Chem. Soc., **2007**, 129 (39), 11918-11919• DOI: 10.1021/ja075736b • Publication Date (Web): 11 September 2007



More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Links to the 1 articles that cite this article, as of the time of this article download
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

View the Full Text HTML

J|A|C|S
COMMUNICATIONS

Published on Web 09/11/2007

Chemoenzymatic Synthesis of Size-Defined Polysaccharides by Sialyltransferase-Catalyzed Block Transfer of Oligosaccharides

Saddam Muthana, Hai Yu, Shengshu Huang, and Xi Chen*

Department of Chemistry, University of California, One Shields Avenue, Davis, California 95616

Received July 31, 2007; E-mail: chen@chem.ucdavis.edu

Sialic acids, a diverse family of nonulosonic acids, have been predominantly found as the outermost carbohydrate units on glycoproteins and glycolipids of vertebrates or on surface polysaccharides of some pathogenic bacteria.[1] The surface polysaccharides (e.g., capsular polysaccharides or lipopolysaccharides) of some pathogenic bacteria also contain internal sialic acid residues, such as *N*-acetylneuraminic acid (Neu5Ac) and 2-keto-3-deoxy-D-glycero-D-galacto-nonulosonic acid (KDN), as part of their oligosaccharide repeating units. For example, *O*-antigen of *Salmonella arizonae* O21 contains [→3)-α-L-Fuc*p*N(1→3)-β-D-Glc*p*NAc(1→7)-α-D-Neu*p*5Ac(2→] as repeating unit.[2] Capsular polysaccharides of *Neisseria meningitidis* group W135 and Y consist of repeating units [→6)-α-D-Gal*p*(1→4)-α-D-Neu*p*5Ac(2→) and [→6)-α-D-Glc*p*(1→4)-α-D-Neu*p*5Ac(2→], respectively.[3] KDN-containing polysaccharides with repeating unit [→2)-α-D-Gal*p*(1→2)-β-D-Rib*f*(1→9)-α-D-KDN*p*(2→] have been detected in the cell wall of *Sinorhizobium freiddi* SVQ293.[4] These ionic polysaccharides are believed to be essential for efficient attachment of pathogenic microorganisms to host cells[5] and are important virulence factors.[6]

Structurally defined polysaccharides containing internal sialic acid residues are extremely difficult to obtain in homogeneous forms either by isolation from natural sources[7] or by chemical synthesis.[8] Developing novel chemoenzymatic approaches to attain size-defined polysaccharides with internal sialic acid residues has been one of our research goals. We report here the first successful example of controlled chemoenzymatic synthesis of size-defined polysaccharides with internal sialic acid residues by sialyltransferase-catalyzed block transfer of oligosaccharide repeating units. The obtained compounds are mimics of naturally occurring polysaccharides.

Sialyltransferases are enzymes that catalyze the transfer of a sialic acid from activated sugar nucleotide donor CMP−sialic acid to an acceptor for the formation of sialic acid-containing structures.[9] The sialyltransferase used here is a recombinant *Photobacterium damsela* α2,6-sialyltransferase (Pd2,6ST).[10] It has flexible donor and acceptor substrate specificities. For example, CMP-Neu5Ac derivatives with diverse modifications on different positions of Neu5Ac are acceptable donors, and compounds with a β-D-galactospyranose (β-D-Gal*p*) at the nonreducing end are good acceptors for the enzyme.[10a,c]

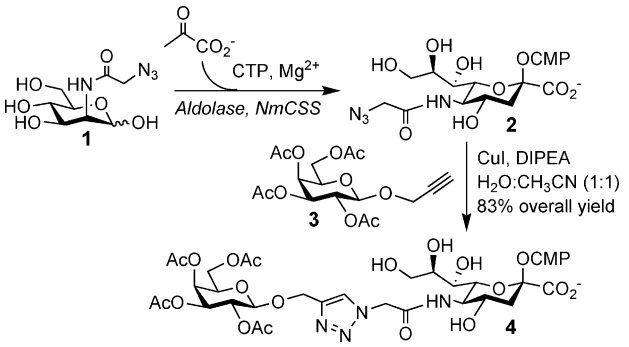
The design for the controlled chemoenzymatic synthesis of polysaccharides with sialic acid-containing repeating units is based on the assumption that sialyltransferases with relaxed substrate specificity can transfer oligosaccharides from their cytidine 5′-monophosphate (CMP)-activated forms to acceptors containing a galactose residue at the nonreducing end (Gal*β*OR). The simplest CMP-activated oligosaccharides that can be potentially used as sialyltransferase donors would be CMP−disaccharides containing a sialic acid that is directly linked to the CMP. If the nonreducing end of the CMP−disaccharides is a β-D-Gal*p*, the product of the sialylation catalyzed by sialyltransferases can be used as an acceptor for the second round of sialylation. Each round of sialylation with CMP−(Gal-Sia) will result in the elongation of the compound with a disaccharide repeating unit (Gal-Sia). When CMP−(Gal-Sia) is intended to be used as a sialyltransferase donor, it can also be considered a β-D-galactoside which can also be recognized by the sialyltransferase as an acceptor. In order to prevent the complication of the sialyltransferase-catalyzed reaction, CMP−(AcGal-Sia) containing a protected Gal, such as a peracetylated galactopyranosyl moiety, at the nonreducing end is used. The designed CMP−(AcGal-Sia) can now only be used as a potential donor for the sialyltransferase. After being transferred to the acceptor, the product can be deprotected and used as an elongated acceptor for another round of sialylation. Repeating the process leads to efficient synthesis of structure- and size-defined polysaccharides.

To test whether oligosaccharides can be transferred from CMP-activated oligosaccharides by Pd2,6ST, the CMP-activated disaccharide analogue **4**, in which the disaccharide analogue moiety consists of a peracetylated galactose linked to a sialic acid residue through a triazole ring, was prepared by an efficient chemoenzymatic approach. As shown in Scheme 1, the azido derivative of CMP−Neu5Ac, CMP−Neu5NAz **2**, was synthesized from *N*-azidoacetyl-D-mannosamine **1** at 37 °C using a one-pot two-enzyme system containing a recombinant *E. coli* sialic acid aldolase and a recombinant *N. meningitidis* CMP−sialic acid synthetase (NmCSS) in Tris-HCl buffer (100 mM, pH 8.5) containing 5 equiv of pyruvate and 1.5 equiv of CTP.[11] After stopping the reaction by adding ethanol to precipitate the protein, the mixture was centrifuged to remove the precipitate and concentrated on a rotary evaporator. The mixture containing **2** was used without purification to react with peracetylated propargyl β-D-galactopyranoside **3** by copper-(I)-catalyzed Huisgen's 1,3-dipolar cycloaddition of azide and alkyne (click chemistry)[12] to yield CMP-activated disaccharide analogue **4** in 83% overall yield after silica gel column and gel filtration column purification.

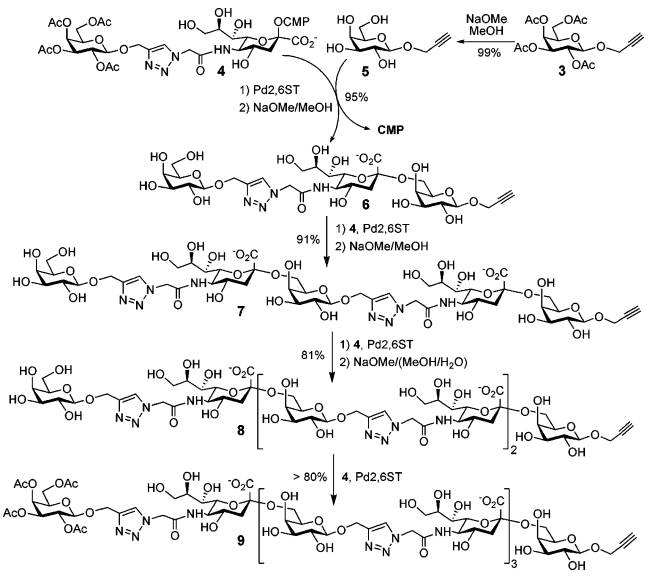
With the desired CMP−disaccharide analogue **4** in hand, we were ready to investigate the suitability of this compound being used as a donor substrate for Pd2,6ST. As shown in Scheme 2, we delightedly found that the compound **4** was an excellent substrate for Pd2,6ST. Pd2,6ST-catalyzed sialylation of propargyl β-D-galactopyranoside **5** in Tris-HCl buffer (100 mM, pH 7.5) followed by deacetylation of the product by Zemplén reaction in sodium methoxide and methanol produced trisaccharide **6** in 95% overall yield. A second round of sialylation using the newly formed trisaccharide **6** as an acceptor followed by deacetylation gave a pentasaccharide **7** in 91% yield. The third round of sialylation and deprotection afforded heptasaccharide **8** in 81% yield. All of these compounds were characterized by high-resolution mass spectrometry (HRMS) and nuclear magnetic resonance (NMR) spectrometry. Tested in small scale and monitored by thin-layer chromatography (TLC), the synthesis of a nonasaccharide **9** from heptasaccharide **8** was also achieved efficiently (>80% yield, estimated by TLC)

10.1021/ja075736b CCC: $37.00

Scheme 1. Chemoenzymatic Synthesis of CMP-Activated Disaccharide Analogue **4**



Scheme 2. Controlled Chemoenzymatic Synthesis of Size-Defined Polysaccharides by Pd2,6ST-Catalyzed Block Transfer of Disaccharides

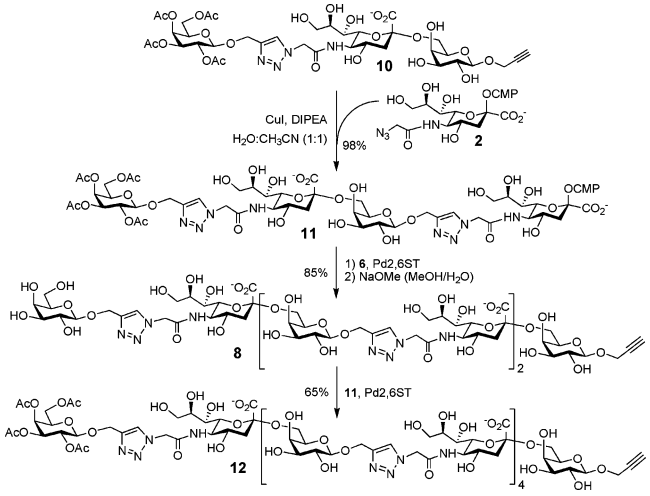


using the similar approach. The formation of the nonasaccharide **9** was confirmed by MALDI-TOF mass spectrometry.

In order to synthesize polysaccharides more efficiently and to further test the flexibility of the Pd2,6ST, a CMP-activated tetrasaccharide **11** was prepared by click chemistry similar to that described for the synthesis of **4**. Briefly, trisaccharide **10** was obtained by Pd2,6ST-catalyzed sialylation of **5** using **4** as a donor followed by silica gel column purification. Copper(I)-catalyzed Huisgen's 1,3-dipolar cycloaddition of the azide in CMP−Neu5NAz **2** and the alkyne in trisaccharide **10** afforded CMP-activated tetrasaccharide **11** in 98% yield. As shown in Scheme 3, the tetrasaccharide in **11** could also be efficiently transferred by Pd2,-6ST to trisaccharide **6** followed by deacetylation to give heptasaccharide **8** in high yield (85%). Second round of sialylation of heptasaccharide **8** using CMP−tetrasaccharide **11** as a donor successfully produced undecasaccharide **12** in good yield (65%). The final product **12** was confirmed by NMR and MS. These results clearly indicate that the newly designed chemoenzymatic synthesis is an efficient and novel approach to obtain size-defined polysaccharides with sialic acid-containing repeating units.

In conclusion, we have demonstrated a novel and highly efficient chemoenzymatic approach for synthesizing structure- and size-defined polysaccharides containing internal sialic acid residues by sialyltransferase-catalyzed block transfer of oligosaccharide repeating units. These compounds are mimics of some surface polysaccharides produced by a number of pathogenic bacteria. The chemoenzymatic synthetic approach reported here provides additional evidence that bacterial sialyltransferases have extremely flexible substrate specificity. They are efficient tools in novel synthesis of diverse and complex sialic acid-containing carbohydrates and glycoconjugates.

Scheme 3. Synthesis of CMP-Activated Tetrasaccharide **11** and Controlled Chemoenzymatic Synthesis of Size-Defined Polysaccharides by Pd2,6ST-Catalyzed Block Transfer of Tetrasaccharides



Acknowledgment. This work was supported by NSF CAREER Award CHE-0548235 and start-up funds from the Regents of the University of California.

Supporting Information Available: Experimental details for enzymatic synthesis, NMR, and HRMS data. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Angata, T.; Varki, A. *Chem. Rev.* **2002**, *102*, 439. (b) Schauer, R. *Glycoconjugate J.* **2000**, *17*, 485.
(2) Vinogradov, E. V.; Paramonov, N. A.; Knirel, Y. A.; Shashkov, A. S.; Kochetkov, N. K. *Carbohydr. Res.* **1993**, *242*, C11.
(3) (a) Bhattacharjee, A. K.; Jennings, H. J.; Kenny, C. P.; Martin, A.; Smith, I. C. *Can. J. Biochem.* **1976**, *54*, 1. (b) Lee, C. H.; Frasch, C. E. *Anal. Biochem.* **2001**, *296*, 73.
(4) (a) Gil-Serrano, A. M.; Rodriguez-Carvajal, M. A.; Tejero-Mateo, P.; Espartero, J. L.; Thomas-Oates, J.; Ruiz-Sainz, J. E.; Buendia-Claveria, A. M. *Biochem. J.* **1998**, *334* (*Pt 3*), 585.
(5) Shashkov, A. S.; Kosmachevskaya, L. N.; Streshinskaya, G. M.; Evtushenko, L. I.; Bueva, O. V.; Denisenko, V. A.; Naumova, I. B.; Stackebrandt, E. *Eur. J. Biochem.* **2002**, *269*, 6020.
(6) Bardotti, A.; Averani, G.; Berti, F.; Berti, S.; Galli, C.; Giannini, S.; Fabbri, B.; Proietti, D.; Ravenscroft, N.; Ricci, S. *Vaccine* **2005**, *23*, 1887.
(7) Chappell, M. D.; Halcomb, R. L. *J. Am. Chem. Soc.* **1997**, *119*, 3393.
(8) (a) Hanson, S.; Best, M.; Bryan, M. C.; Wong, C. H. *Trends Biochem. Sci.* **2004**, *29*, 656. (b) Daines, A. M.; Maltman, B. A.; Flitsch, S. L. *Curr. Opin. Chem. Biol.* **2004**, *8*, 106. (c) Kiefel, M. J.; von Itzstein, M. *Chem. Rev.* **2002**, *102*, 471.
(9) HarduinLepers, A.; Recchi, M. A.; Delannoy, P. *Glycobiology* **1995**, *5*, 741.
(10) (a) Yu, H.; Huang, S.; Chokhawala, H.; Sun, M.; Zheng, H.; Chen, X. *Angew. Chem., Int. Ed.* **2006**, *45*, 3938. (b) Yamamoto, T.; Nakashizuka, M.; Terada, I. *J. Biochem. (Tokyo)* **1998**, *123*, 94. (c) Kajihara, Y. Y., T.; Nagae, H.; Nakashizuka, M.; Sakakibara, T.; Terada, I. *J. Org. Chem.* **1996**, *61*, 8632.
(11) Yu, H.; Yu, H.; Karpel, R.; Chen, X. *Bioorg. Med. Chem.* **2004**, *12*, 6427.
(12) (a) Huisgen, R. *Angew. Chem., Int. Ed. Engl.* **1963**, *2*, 633. (b) Rostovtsev, V. V.; Green, L. G.; Fokin, V. V.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2002**, *41*, 2596. (c) Tornøe, C. W.; Christensen, C.; Meldal, M. J. *J. Org. Chem.* **2002**, *67*, 3057. (d) Bodine, K. D.; Gin, D. Y.; Gin, M. S. *J. Am. Chem. Soc.* **2004**, *126*, 1638.

JA075736B