Recent Synthetic Approaches to Inner-core Oligosaccharides of Lipopolysaccharides and Lipooligosaccharides

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(Received on November 14, 2017, accepted on December 7, 2017)

Key Words: lipopolysaccharide, lipooligosaccharide, inner core, 3-deoxy-D-manno-oct-2-ulonic acid

Abstract

Lipopolysaccharides (LPSs) and lipooligosaccharides (LOSs) are target molecules for the development of vaccines against pathogenic Gram-negative bacterial infections. The chemical synthesis of the core oligosaccharides of LPSs/LOSs remains a major challenge. Herein, recent reports of the chemical synthesis of inner-core oligosaccharides containing 3-deoxy-D-manno-oct-2-ulonic acid are reviewed.

A. Introduction

Lipopolysaccharides (LPSs) and lipooligosaccharides (LOSs) are glycolipids found in the outer membranes of Gram-negative bacteria. They are not only essential for maintaining their life but well known for their immunological, pharmacological and pathophysiological effects displayed in eukaryotic cells and organisms (Fig. 1). Alongside capsular polysaccharides, pili, and extracellular proteins, LPSs and LOSs are important targets in the biological defense response against bacterial infection and their study is vital for elucidating the mechanism of this response and developing vaccines.

LPSs are composed of three components, namely, lipid A, a core oligosaccharide, and an O-antigen polysaccharide, whereas LOSs lack the O antigen. The core oligosaccharide can be divided into inner core and outer core regions, where the inner core is made up of the high-carbon sugars 3-deoxy-D-manno-oct-2-ulonic acid (Kdo) and L-glycero-D-mannoheptose (Hep). As these sugars are not found in mammals, they have received attention for their potential use as antigens in vaccines against pathogenic Gram-negative bacteria.

The structures of the inner-core oligosaccharides of LPSs and LOSs have rich diversity but tend to be highly conserved within a genus. The biological function of the inner core has not yet been clarified. Investigations into the chemical synthesis of inner-core oligosaccharides began in the mid-1980s, although their construction remains challenging. This is because the two main components, Kdo and Hep, are not readily available for the synthesis, and the stereoselectivity of glycosylation using Kdo donors cannot be easily controlled.

Biosynthesis of glycans of LPS is also complicated as well as other glycans due to primarily determined by the glycosyltransferases that assemble monosaccharide moieties into linear and branched glycan chain. Therefore it is difficult to obtain the inner-core oligosaccharides of LPSs and LOSs in a pure form through a molecular biological approach. To circumvent this problem, chemical methods have been applied to achieve the synthesis of inner-core oligosaccharides. This mini review summarizes the latest advances in the chemical synthesis of Kdo-containing inner-core oligosaccharides of LPSs and LOSs.

B. Synthesis of Kdo-containing LPS Inner-core Oligosaccharide

Zamyatina *et al.* reported the synthesis of a disaccharide containing 4-amino-4-deoxy-L-arabinose (Ara4N), which is part of the inner-core oligosaccharide of the LPS produced by *Burkholderia* sp. (1). (Scheme 1). Glycosylation of the *N*-phenyltrifluoroacetimidate **1** derived from 2,3-di-*O*-benzyl 4-azido-4-deoxy-L-arabinose with the D-glycero-D-taro-oct-2-ulonic acid (Ko) derivative **2** afforded the 1,8- and 1,7-linked disaccharides (**3a,b** and **4**) in 84% yield with moderate regio- and poor stereoselectivity. The separation of these products was easily achieved after removal of the α 1-8 linked disaccharide and subsequent deacetylation of the β -1,8

Fig. 1. Biological activity of LPS.

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a) organism level	pyrogenicity, leucopenia, thrombocytopenia, hypoglycemia, hypoferrremia, hypotensive action, intravascular coagulation, immunologic enhancement
b) cell level	macrophage activation, macrophage migration inhibition, lymphocyte mitogenic activation, prostaglandin production, anti-tumor activity, microglia activation
c) molecular level	complement activation, cytokine production, plasminogen activation
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Scheme 1. Synthesis of Ara4N-(1-8)-Ko disaccharide. Reagents and conditions: a) TMSOTf, -25°C , b) i) TiCl_4 , 0°C , ii) Ac_2O 89%, c) i) NaOMe , ii) NaOH , 97%. d) DTT, NH_3 , 60%.

and β -1,7 derivatives under Zemplén conditions. In another study, glycosylation of the 8-OH position of the Kdo derivative **8** afforded the core disaccharide **12** of the LPSs from *Proteus mirabilis* and *Proteus penneri* (Scheme 2). In addition, glycosylation of triol **9** with Kdo bromide **13** under Helferich conditions gave a branched core trisaccharide β -Ara4N-(1-8)- α -Ko-(2-4)-Kdo **17** of the LPS from *Proteus mirabilis*.

Pokorny *et al.* reported the synthesis of the inner-core trisaccharide and branched tetrasaccharide of the *Chlamydia* LPS using the 3-iodo-Kdo fluoride **18** as the glycosyl donor (**2**) (Scheme 3). One-pot glycosylation of the Kdo donor **18** and tetraol acceptor **19** afforded the desired Kdo trisaccharide **20**, α -Kdo-(2-4)[α -Kdo-(2-8)]-Kdo, as the major product in 58% yield, alongside its regioisomer, α -Kdo-(2-4)[α -Kdo-(2-7)]-Kdo, in 7% yield. This result seems to indicate that the reactivity of the hydroxyl groups of Kdo derivatives is in the order of 4-OH > 8-OH > 7-OH > 5-OH. In these glycosylation reactions, a Kdo donor is less reactive than other neutral aldose-type donors because of steric and electronic factors arising from the C1 carboxyl group in the Kdo donor. In addition, control of the stereoselectivity under glycosylation conditions is

very difficult due to the lack of a neighboring functional group at the C3 position; however, the use of 3-iodo-Kdo fluoride as the donor solves these problems. By applying this method, the first synthesis of an α -2,5 linked Kdo dimer **24** containing the inner core of the *Acinetobacter* LPS was achieved (**3**) (Scheme 4).

Yang *et al.* reported the synthesis of the inner-core tetrasaccharides of LPSs from *Yersinia pestis*, *Haemophilus influenzae*, and *Proteus* sp. using the disaccharide α -L,D-Hep-(1-3)- α -L,D-Hep-(1-5)-Kdo derivative **25** as a common building block (**4**, **5**) (Scheme 5). The key features of this synthesis are (a) preparation of Hep units with various protecting groups by *de novo* synthesis from Hep derivatives, and (b) glycosylation of the Kdo derivative **25** at the 4-position with the oligosaccharide donors **26–28** prepared from these protected Hep units to afford a variety of oligosaccharide structures **29–31**. In this synthesis of **25**, the acetyl protecting group at the 2-position of the reducing Hep unit of the donor was considered to have a neighboring-group effect (**6**).

Huang *et al.* designed a 5,7-*O*-di-*tert*-butylsilylene (DTBS)-protected Kdo ethyl thioglycoside as a Kdo donor. Glycosylation using this donor with primary and secondary glycosyl acceptors

Scheme 2. Synthesis of *P. mirabilis* and *P. Penneri* di- and trisaccharide. Reagents and conditions: a) **33**, TMSOTf, -25°C , b) Ac₂O, ii) TiCl₄, 0 $^{\circ}\text{C}$, iii) Ac₂O, (68%, **11**) (51%, **16**). c) i) NaOMe, ii) NaOH (98%, **12**), (96%, **17**). d) DTT, NH₃ 60%. e) Hg(OAc)₂, CH₃NO₂, 19%. f) Me₃P, THF, 99%.

Scheme 3. Synthesis of the family-specific *Chlamydia* Kdo trisaccharide. Reagents and conditions: a) BF₃·OEt₂, CH₂Cl₂, MS 3A 58%. b) Ac₂O, Pyridine, DMAP, 95%. c) lauroyl peroxide, cyclohexane 88%. d) 0.1 M NaOMe, MeOH, then 0.1 M aq. NaOH 71%.

gave the corresponding α -glycosides in moderate to excellent yields with complete selectivity (**7**). To demonstrate the utility of this approach, they applied it to the synthesis of the 4,8-branched trisaccharide present in the core structure of *Burkholderia* and *Proteus* LPSs. Glycosylation of allyl alcohol with thioglycoside **32** afforded α -allyl glycoside **33** as the sole product (Scheme 6). Subsequently, glycosylation with Ara4N donor **1** gave the corresponding 1,8-linked disaccharides **35a,b** in excellent yield with low stereoselectivity (1:1.5). After separation of these diastereomers, the disaccharide **35a** was coupled with the Kdo donor **32** to

deliver the 4,8-branched trisaccharide **37** in good yield. In addition, the synthesis of a common LPS epitope of the human pathogen *Chlamydia*, α -Kdo-(2-8)- α -Kdo-(2-4)- α -Kdo trisaccharide, was also demonstrated.

Boltje *et al.* reported that the disaccharide **38** modified by the orthogonal protecting groups diethylisopropylsilyl (DEIPS), 2-naphthyl (Nap), allyl (All), and levulinoyl (Lev) can serve as a reliable key compound for the synthesis of the inner core of LPSs (**8**) (Scheme 7). The ability to selectively remove the various protecting groups in the desired order allowed the optimal coupling

Scheme 4. Glycosylation and deprotection towards the α -(2-5) Kdo disaccharide. Reagents and conditions: a) $\text{BF}_3 \cdot \text{OEt}_2$, toluene, -40°C to -15°C , 2 h, -15°C to -10°C , 1.5 h, 71%. b) i) $\text{Pd}(\text{OH})_2/\text{C}$, H_2 , MeOH, NaOAc, rt, 1 h, ii) Pd/C , H_2 , MeOH, rt, 20 h, iii) Ac_2O , DMAP, pyr, rt, 24 h, 61%; c) i) NaOMe MeOH, rt, 16 h, ii) NaOH, H_2O , 0°C , 2 h then rt 4 h, 90%.

Scheme 5. Synthesis of the inner-core oligosaccharides 26–28. Reagents and conditions: TMSOTf, 4A MS, CH_2Cl_2 , Et_2O , 0°C , to rt, 73%, **26**, 67%, **27**, 65%, **28**.

sequence to be established for the construction of a highly crowded 1,2,3-*cis* configured branching point. Glycosylation of **38** using the GalN derivative **39** followed by selective cleavage of the DEIPS

Scheme 8. Synthesis of the inner-core tetrasaccharide of *Neisserial* LOS. Reagents and conditions: a) TMSOTf, DCM, 45%. b) NaOMe, MeOH. c) TBDPSCl, pyridine, 79%. d) NaH, BnBr, DMF, 57%. e) TBAF, THF, 88%. f) i) COCl₂, DMSO, Et₃N, DCM. ii) vinyl MgBr, THF, 48% in two steps. g) NaH, BnBr, DMF, 70%. h) i) OsO₄, NMO, acetone/water, 71%. ii) NaIO₄, MeOH/water, 79%. iii) NaBH₄, MeOH/water, 91%. i) Pd/C, H₂, EtOH, 93%. ii) Ac₂O, Pyridine, 76%. j) HBr/AcOH, 80%. k) BF₃·OEt₂, DCM, 0°C, 25%. l) AgOTf, DTBMP, DCM. m) TFA/H₂O (3:2), DCM. n) i) Pd/C, H₂, EtOH: water = 1:1. ii) 1 M NaOH, 34% over 4 steps.

Kdo unit **41** gave the linked tetrasaccharide **42**. Coupling with glucose donor **43** afforded β -glycoside **44** after selective deprotection of the levulinoyl group. Finally, oxidative cleavage of the naphthyl group followed by β -selective glucosylation with glucose donor **45** and full deprotection afforded the inner core of the LPS from *Francisella tularensis*.

Kong *et al.* reported the synthesis of the inner-core tetrasaccharide of the *Neisseria* LOS by the coupling of a Kdo dimer with a Hep dimer (**9**) (Scheme 8). Disaccharide **49**, α -Hep-(1-3)-Hep, was prepared from α -(1-3)-mannobiose derivative **48** by oxidation of the two hydroxymethyl groups to aldehydes followed by two-carbon elongation using vinylmagnesium bromide and oxidative cleavage of the resulting vinyl group. The diastereoselectivity of the addition reaction was moderate. Coupling reactions with the heptosyl bromide, ethylthio-, trichloroacetimidoyl, and *N*-phenyl-trifluoromethylimidoyl derivatives of **49** were attempted, but only the use of the glycosyl bromide was successful, affording tetrasaccharide **50** in 25% yield ($\alpha:\beta=9:1$).

We reported the synthesis of a core oligosaccharide containing a 4,5-branched Kdo structure *via* fragment condensation. This approach involves the coupling at the 5-OH position of α -2,4 linked Kdo acceptor **51** with monosaccharide and oligosaccharide donors. Table 1 shows the results of the glycosylation. The use

of mannosyl or heptosyl trichloroacetimidates (**52** and **53**) gave the corresponding α -linked trisaccharides (**57** and **58**) as the sole products (**10**). The glycosylation using oligosaccharide donors **55** and **56** also proceeded smoothly to afford the corresponding 4,5-branched glycosides in good yield and excellent stereoselectivity (**11**). This was the first synthesis of a continuously vicinal diol branched structure that contains the core oligosaccharide produced by *Neisseria* and found in other bacterial LPSs and LOSs.

C. Conclusions

Over the past decade, chemical synthesis has been used to obtain a number of interesting inner-core oligosaccharides in high purity. It is expected that additional types of inner-core oligosaccharides will be synthesized in the future, allowing the immunological and biological functions and roles of inner-core oligosaccharides to be elucidated.

Acknowledgments

The author is grateful to Ms. Maki Taniguchi for technical assistance and would also like to thank his collaborators for their contributions to the project, which was supported by JSPS KAKENHI grants (JP23580474 and JP15K01822).

Table 1. Synthesis of 4,5-branched and continuously branched inner-core oligosaccharide.

<p>Reaction scheme: Donor + Acceptor $\xrightarrow[\text{CH}_2\text{Cl}_2, \text{MS AW-300}]{\text{TMSOTf (0.06 eq)}}$ Product 51</p>	
Donor	Product
<p>52</p>	<p>57 Hep 87%</p> <p>58 Man 91%</p> <p>59 GalN₃ 57%</p>
<p>53</p>	
<p>54</p>	
<p>55</p>	
<p>56</p>	<p>60</p>
	<p>61</p>

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Tsuyoshi Ichiyanagi received his Dr. Eng. from the Graduate School of Mie University in 1998 under the supervision of Professor Tamotsu Fujisawa. He then moved to Tohoku University, where he worked as an Instructor at the Institute of Chemical Reaction Science (1998–2000). In 2000, he moved to RIKEN as a Postdoctoral Fellow of the Japan Science and Technology Agency under direction of Dr. Yoshiaki Nakahara (2000–2001), and later became a Special Postdoctoral Researcher in Synthetic cellular chemistry laboratory with Dr. Yukishige Ito at RIKEN (2001–2002). In 2002, he moved to Tottori University as a Lecturer, where he became an Associate Professor in 2007 and a Full Professor in 2016. His main research interest is synthetic organic chemistry, focusing on carbohydrates and natural products from fungi.