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RESEARCH ARTICLE

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Chemical Synthesis and Immunological Evaluation of *Helicobacter pylori* Serotype O6 Tridecasaccharide O-Antigen Containing a Unique DD-Heptoglycan

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Dedicated to the 100th anniversary of the birth of Prof. Dr. Raymond Urgel Lemieux

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Abstract: The development of glycoconjugate vaccines against *Helicobacter pylori* is challenging. An exact epitope of the *H. pylori* lipopolysaccharides (LPS) O-antigens that contain Lewis determinant oligosaccharides and unique DD-heptoglycans has not yet been identified. Here, we report the first total synthesis of *H. pylori* serotype O6 tridecasaccharide O-antigen containing a terminal Le^y tetrasaccharide, a unique α-(1→3)-, α-(1→6)- and α-(1→2)-linked heptoglycan, and a β-D-galactose connector through an [(2 × 1) + (3 + 8)] assembly sequence, which may serve as reference for the syntheses of other sterically hindered long-chain glycans. Seven oligosaccharides covering different portions of the entire O-antigen were prepared for immunological investigations with a particular focus on elucidation of the roles of DD-heptoglycan and Le^y tetrasaccharide. Glycan microarrays analysis of sera from rabbits immunized with isolated serotype O6 LPS revealed a humoral immune response to the α-(1→3)-linked heptoglycan, which is the key motif for designing glycoconjugate vaccines for *H. pylori* serotype O6.

Introduction

Helicobacter pylori infects half of the world's population,[1] and is strongly associated with chronic gastritis, peptic ulcer and gastric cancer.[2-4] Infections with this bacterium increase the risk for developing gastric cancer two- to eightfold,[5–8] and led to its classification as a class I carcinogen.[9] Combined antimicrobial and anti-acid therapy can eradicate *H. pylori* infections,[10] but the overuse of currently recommended treatments has resulted in antimicrobial resistance.[11] Antimicrobial cure of *H. pylori* infections does not prevent re-infection, such that vaccines are urgently needed.[12,13] Despite significant efforts and a fusion protein-based vaccine consisting of urease B subunit and heat-labile enterotoxin B subunit licensed in China since 2009,[14] no *H. pylori* vaccine is marketed globally today.

Lipopolysaccharide (LPS) O-antigens, a major cell surface component of *H. pylori*, have been recognized as attractive vaccine candidates.[15,16] The structures of LPS O-antigens from six known *H. pylori* serotypes (O1 to O6) and various untypable *H. pylori* strains have been reported.[17-19] A series of unique DD-heptoglycans consisting of seven to thirteen D-glycero-α-D-manno-heptopyranoses (DD-heptoses) and three different glycosidic linkages α-(1→2), α-(1→3) and α-(1→6) (Figure 1) has been found in *H. pylori* serotype O3,[20] O5,[16] O6,[20] strain MO19[21] and a number of untypable *H. pylori* strains isolated from asymptomatic hosts.[18] Until now, other glycans comprising solely DD-heptose was only found in the LPS outer core region of *Klebsiella pneumoniae* strain 01/R20 that has been elucidated as an α-(1→2)-linked DD-heptoglycan.[22] Most *H. pylori* LPS O-antigens (O1, O3, O4, O5 and O6) are terminated by oligosaccharides resembling mammalian histo-blood-group antigens, such as Lewis determinants (Le^a, Le^b, sialyl Le^x, Le^x and Le^y).[17] These mammalian histo-blood-group antigen analogues have not been found in untypable *H. pylori* strains from asymptomatic hosts.[18,19] Lower pathogenicity of *H. pylori* strains lacking Lewis determinant oligosaccharides suggests the notion that the extended heptoglycan region camouflage *H. pylo-*

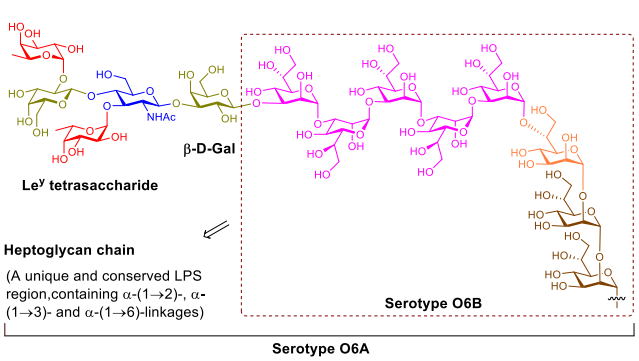


Figure 1. Structure of O-antigen of *H. pylori* serotype O6 LPS.

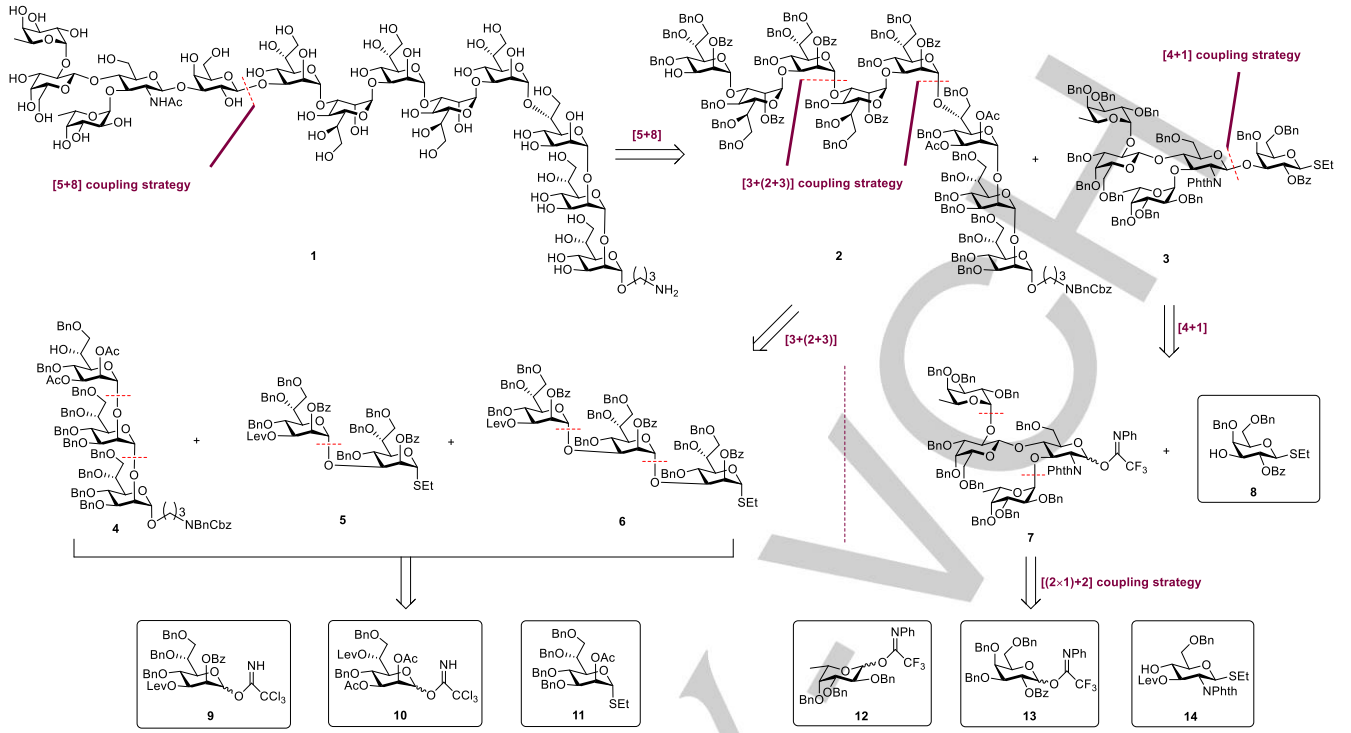
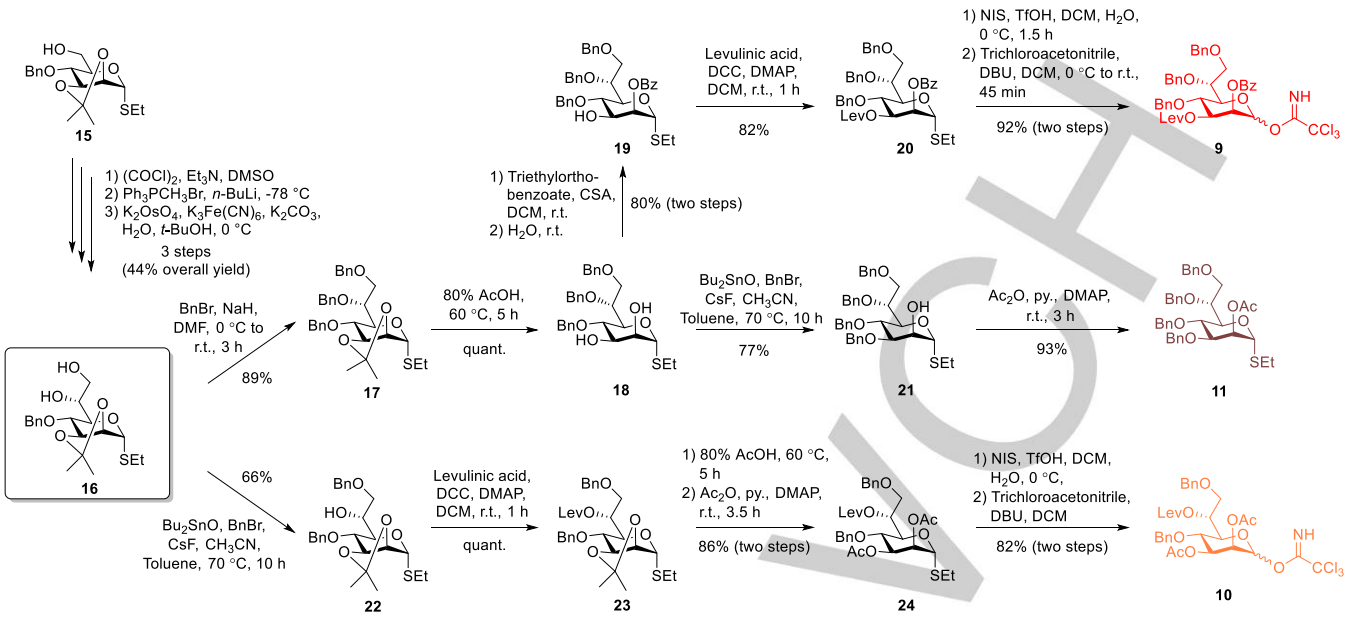


Figure 2. Retrosynthetic analysis of O-antigen tridecasaccharide of *H. pylori* serotype O6.

ri for immune escape.[17,18] On the other hand, several studies suggested the existence of specific epitopes sharing a common structural motif located in *H. pylori* O-antigens, since patients' sera can recognize immunogenic LPS region, rather than Lewis antigen analogues.[23,24] The roles of Lewis determinant oligosaccharides and heptoglycans in the *H. pylori*-immune system interactions remain controversial due to lack of molecular understanding. Structurally-defined carbohydrate antigens are required to establish the roles of these two oligosaccharide portions of *H. pylori* O-antigens.[25] Chemical synthesis offers an attractive means to procure the specific carbohydrate antigens needed to elucidate their roles in *H. pylori* pathogenicity and advance the development of glycoconjugate vaccines.[26] Various Lewis determinant oligosaccharides have been chemically[27] and enzymatically[28] synthesized. The DD-heptoglycan has not yet been synthesized. The O-antigen of *H. pylori* serotype O6, that is divided into two subtypes O6A and O6B,[20] is a representative glycan containing both Lewis determinant oligosaccharides and DD-heptoglycan. The *H. pylori* O6A O-antigen tridecasaccharide is composed of a terminal Le^y tetrasaccharide and a DD-heptoglycan consisting of eight DD-heptoses (two α-(1→2)-, four α-(1→3)- and one α-(1→6)-glycosidic linkages), that are connected via a β-D-galactose residue (Figure 1).[20] *H. pylori* serotype O6B expresses a truncated O-antigen consisting solely of the DD-heptoglycan (Figure 1).[20] The chemical synthesis of the *H. pylori* O6A O-antigen tridecasaccharide and O6B O-antigen octasaccharide is essential for immunological identification of *H. pylori*-expressing Le^y tetrasaccharide and DD-heptoglycan. The chemical synthesis of *H. pylori* O6 O-antigen tridecasaccharide poses a number of challenges, especially the steric hindrance of DD-heptoglycan that influences glycosylation efficiencies. To improve the yield of glycosylation reactions, an electronic effect-based protecting group strategy,[29,30] optimization of leaving group/activator condition[31] and steric effect-based assembly sequence planning[32-34] served well to tune the reactivities of the glycosyl donor and acceptor. Here, we report the first total synthesis of *H. pylori* serotype O6A and O6B O-antigens by a trial-and-error approach based on the reactivities of the glycosyl donor and acceptor, to prepare sterically hindered long-chain glycans. Glycan array screening identified the key epitopes that can be recognized by IgG antibodies against native LPS as a basis for the design of synthetic carbohydrate conjugate vaccines.[35] Therefore, a library of synthetic serotype O6 O-antigen fragments has been established for immunological investigations, particularly to elucidate the antigenicity of the Le^y oligosaccharide and DD-heptoglycan.

Results and Discussion

Retrosynthetic Analysis. An aminopropyl linker was placed at the reducing end of synthetic tridecasaccharide **1** for immobilization on glycan array surfaces or conjugation to carrier proteins (Figure 2). The assembly of long-chain glycan **1** relies on the convergent assembly of two judiciously selected oligosaccharide fragments. Considering the steric hindrance of DD-heptoglycan, the final assembly point should not reside within the DD-heptoglycan part. Thus, retrosynthetic analysis initiated from a [5 + 8] coupling of a pentasaccharide **3** and a hepto-octasaccharide **2**. For the assembly of octasaccharide **2**, a [3 + (2 + 3)] coupling from the reducing to the nonreducing end was adopted, but not a [(3 + 2) + 3] coupling from the nonreducing to



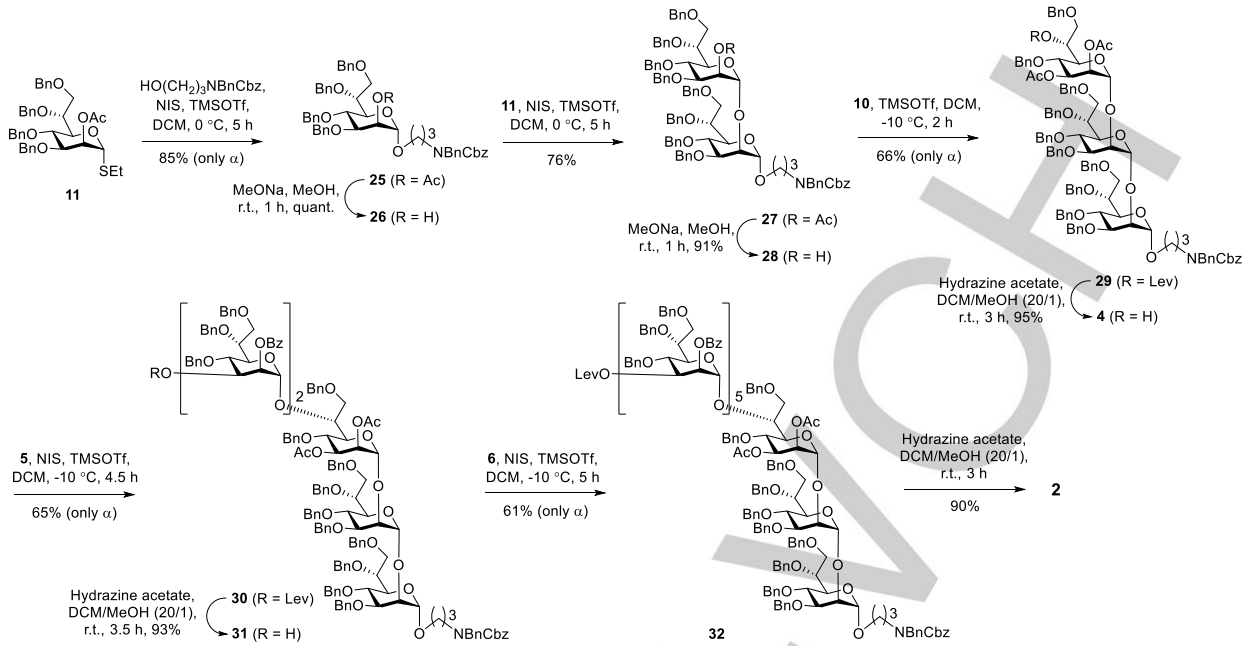
Scheme 1. Diversity-oriented synthesis of heptosides **9**, **10** and **11**.

the reducing end mainly due to worries over the low reactivity of the large glycosyl donor. Consideration of the orthogonal protecting groups were required for regioselective glycosylation, three building blocks **9**, **10** and **11** were identified for the assembly of heptoglycans. Making use of previous syntheses of Le^y tetrasaccharide,[27] pentasaccharide **3** was designed to be prepared via coupling of Le^y tetrasaccharide donor **7** and thio-galactoside **8**. A [(2 × 1) + 2] coupling strategy was chosen to assemble Le^y-tetrasaccharide from three building blocks **12**, **13** and **14**.

Synthesis of Manno-Heptosides 9, 10 and 11. A diversity-oriented synthesis of manno-heptosides **9**, **10** and **11** started with thioglycoside **15**[36] (Scheme 1). Key heptoside **16** bearing two free hydroxyl groups was obtained from **15** in an overall yield of 44%. Treatment of **16** with benzyl bromide (BnBr) and sodium hydride (NaH) in dimethylformamide (DMF) afforded benzylated thioglycoside **17**. The isopropylidene group in **17** was smoothly removed under acidic condition to produce diol **18** that was employed for the divergent synthesis of manno-heptosides **9** and **11**. An *O*2 regioselective benzoylation of **18** via an orthoester intermediate afforded **19** in 80% overall yield. Next, treatment with levulinic acid and dicyclohexylcarbodiimide (DCC) converted **19** to fully protected thioglycoside **20** in 82% yield. The hydrolyzed product of **20** was transformed to Schmidt donor **9** that can be glycosylated with thioglycoside acceptors. The tin-mediated regioselective benzylation at *O*3 of the diol **18** furnished **21** that was converted to the *O*2 acetylated thioglycoside **11** in 72% overall yield. To synthesize the third manno-heptoside **10**, regioselective *O*7 benzylation of diol **16** produced 7-OBn product **22** in 66% yield. The *O*6 protection of compound **22** by using levulinic acid and DCC gave 6-*O*-Lev derivative **23** quantitatively. Subsequent removal of the isopropylidene group and acetylation furnished **24**, which was transformed to Schmidt donor **10** in good overall yield.

Synthesis of Heptoglycan-Octasaccharide 2. With three manno-heptosides **9**, **10** and **11** in hand, the assembly of octa-heptan **2** started from the reducing to the non-reducing end (Scheme 2). As the first step, a propylamine linker was introduced to the anomeric position of thioglycoside **11** to afford **25** in 85% yield as the sole isomer. After removal of the *O*2 acetyl group, desired glycosyl acceptor **26** was coupled with thioglycoside **11** to produce 1→2-linked di-heptan **27** in 76% yield with exclusive α-selectivity. Cleavage of an acetyl group converted **27** to alcohol **28** that was reacted with thioglycoside **24** to give 1→2-linked tri-heptan **29** along with inseparable byproducts. Glycosylation of **28** with Schmidt donor **10** promoted by trimethylsilyl trifluoromethanesulfonate (TMSOTf) smoothly furnished tri-heptan **29** in 66% yield with exclusive α-selectivity. Next, to enhance the glycosylation efficiencies of the penta-heptan containing four 1→3-linked heptosidic bonds, manno-heptoside **9** was used to prepare two donors: dimer **5** and trimer **6** (for detailed syntheses please see Supporting Information). A subsequent [3 + (2 + 3)] coupling starting from the delevulinated tri-heptan **4**, rather than a [(3 + 2) + 3] coupling, provided an easy entry to the assembly of octa-heptan **2**. Thus, the tri-heptan **4** was smoothly coupled with dimer **5** to afford penta-heptan **30** containing 1→6-linked heptosidic bond. After removal of levulinyl group, acceptor **31** was glycosylated with trimer **6** under NIS and TMSOTf promotion at -10 °C to produce octa-heptan **32** in good yield and complete α-selectivity. The further delevulination gave the desired glycosyl acceptor **2** in 90% yield.

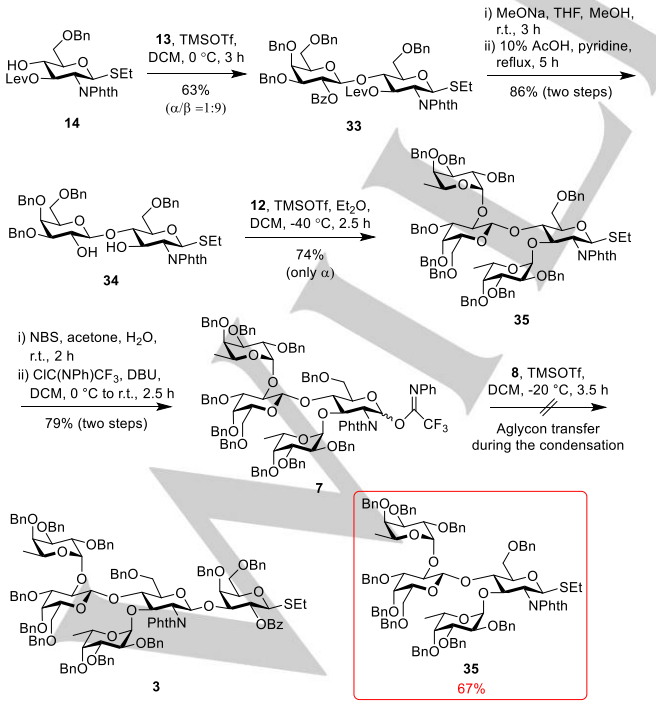
Synthesis of Le^y-Containing Pentasaccharide. The synthesis of the Le^y tetrasaccharide started from the coupling of building block **14** (see Supporting Information) and trifluoroacetimidate glycosyl donor **13** (see Supporting Information) catalyzed by TMSOTf, producing disaccharide **33** in 63% yield with an α/β ratio of 1:9 (Scheme 3). Cleavage of the benzoyl and levulinyl groups in **33** with sodium methoxide treatment afforded a phthalimide ring-opened diol intermediate, which was refluxed in



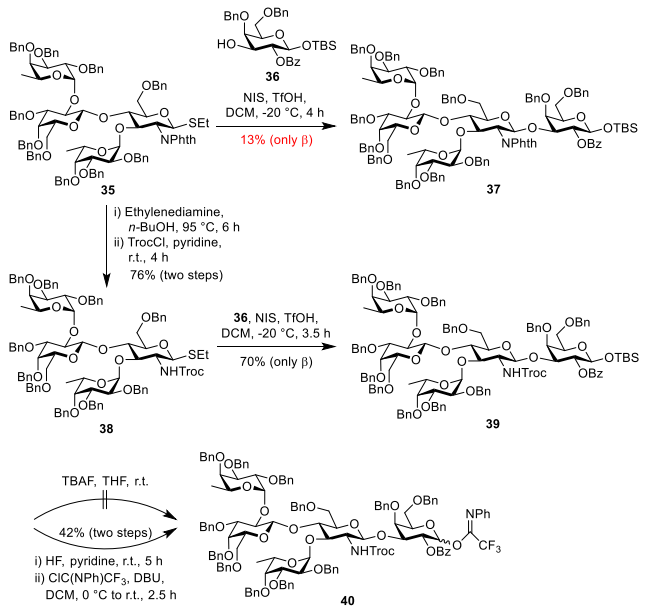
Scheme 2. Synthesis of heptoglycan-octasaccharide **2**.

pyridine and 10% aqueous AcOH to give acceptor **34** in 86% overall yield. Condensation of disaccharide **34** with L-fucosyl donor **12**[37] under the TMSOTf activation in a mixture of Et_2O and CH_2Cl_2 at -40 °C gave Le^y tetrasaccharide **35** in 74% yield and complete α-selectivity. Thioglycoside **35** was transformed to the corresponding *N*-phenyl trifluoroacetimidate glycosyl donor **7** that can be glycosylated with thioglycoside acceptors. Synthesis of the pentasaccharide using tetrasaccharide donor **7** and thioglycoside acceptor **8** (see Supporting Information) failed, but gave mainly a by-product identified as **35**. Likely, aglycon transfer occurred under the TMSOTf-catalyzed glycosylation conditions due to unmatched reactivities between the glycosyl donor and acceptor.[38]

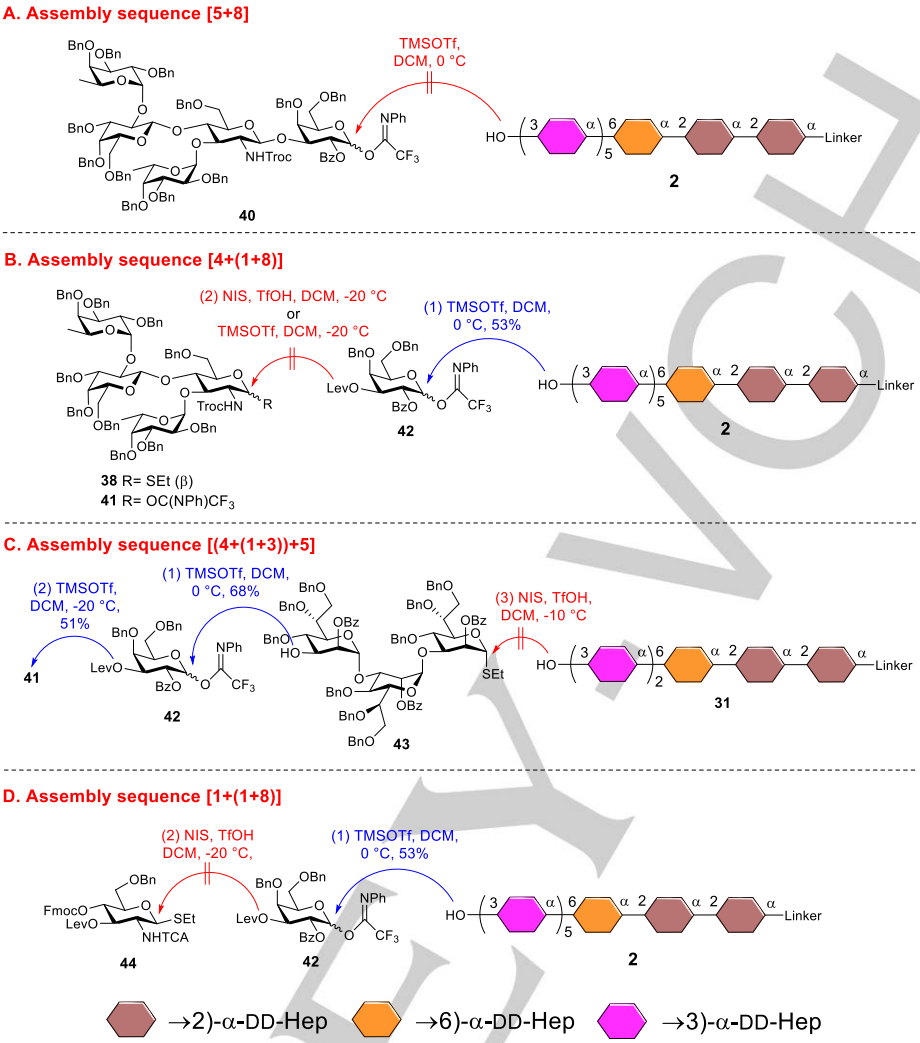
To address the problem of aglycon transfer, thioglycoside **8** was transformed to the *O*1-TBS compound **36** (see Supporting Information) (Scheme 4). Unfortunately, the glycosylation of thioglycoside **35** and acceptor **36** afforded pentasaccharide **37** in low yield (13%). D-Glucosamine derivatives show different glycosyl donor reactivities depending on the protecting group pa-



Scheme 3. Attempt to Le^y-containing pentasaccharide.



Scheme 4. Synthesis of Le^y-containing pentasaccharide **40**.

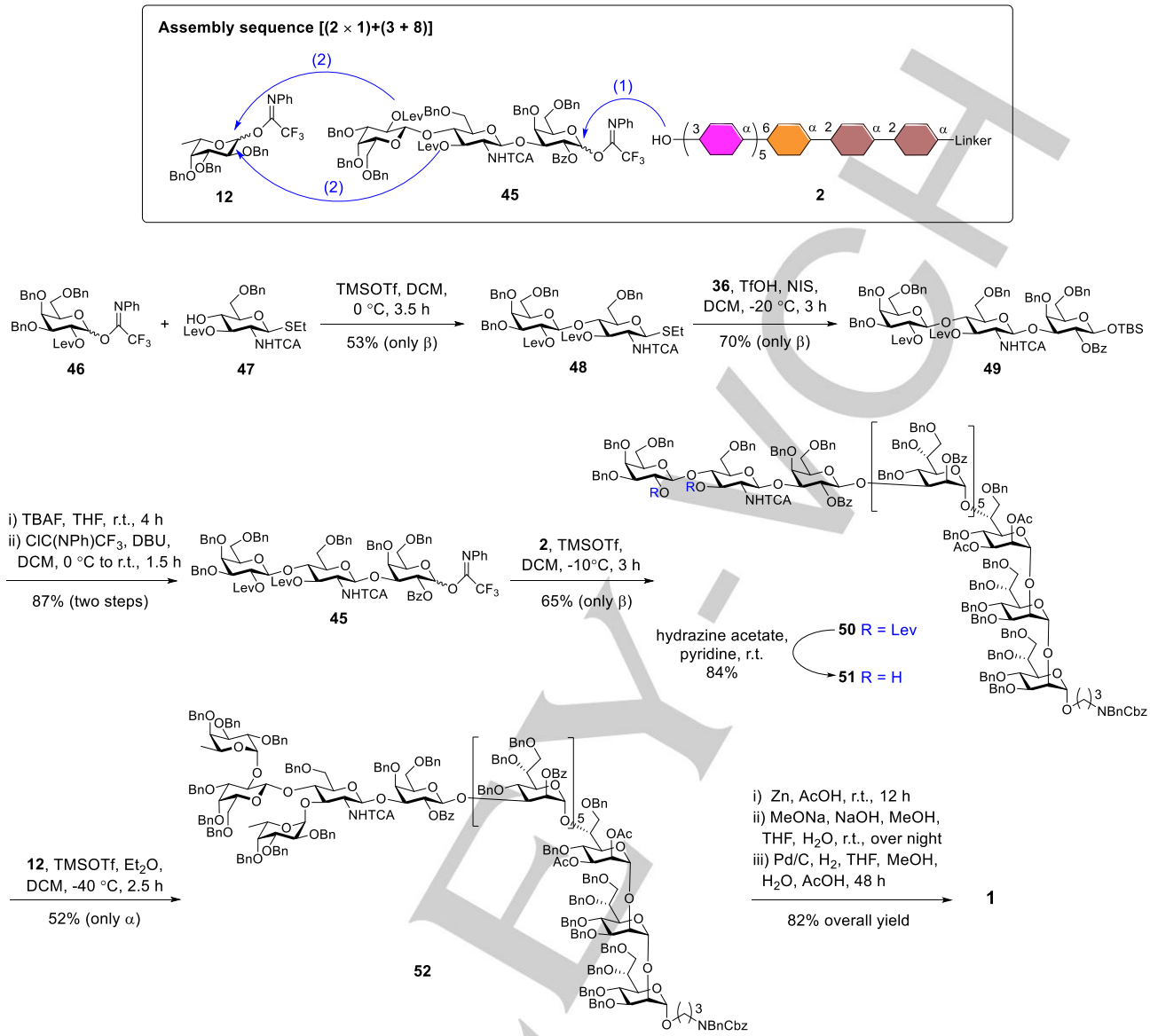


Scheme 5. Attempted assembly sequence for the synthesis of serotype O6 O-antigen tridecasaccharide.

tterns of 2-NH_2: *N*-2,2,2-trichlorethoxycarbonyl-(Troc) > *N*-phthalimido-(Phth) > azido- > *N*-acetyl-.[29] Since the relative reactivity value (RRV) (28.6) of 2-NHTroc-D-glucosamine donor is higher than that (1.0 - 3.5) of the 2-NPhth-D-glucosamine donor,[29] Troc was chosen as an alternative protecting group for the C2 amino group of Le^y tetrasaccharide. Selective removal of the Phth group using ethylene diamine in *n*-BuOH at an elevated temperature (95 °C)[39] and subsequent treatment with TrocCl in pyridine furnished the tetrasaccharide **38**. Glycosylation of donor **38** and acceptor **36** under TfOH and NIS activation at -20 °C afforded the β-linked product **39** in 70% yield. Partial cleavage of Troc under TBAF conditions forced us to choose HF-pyridine to remove anomeric TBS group. The obtained hemiacetal was transformed to the corresponding trifluoroacetimidate **40** in 42% overall yield.

Synthesis of *H. pylori* O6 O-Antigen Tridecasaccharide 1 and Related Oligosaccharides. The tridecasaccharide was assembled via a [5 + 8] coupling (Scheme 5A). Unfortunately, the glycosylation of octasaccharide acceptor **2** and pentasaccharide donor **40** in the presence of TMSOTf at 0 °C failed to give the target tridecasaccharide. Unreacted acceptor and donor degradation products suggested that the failure of the glycosylation reaction may be due to steric hindrance in octasaccharide acceptor **2**. In view of the successful coupling of tetrasaccharide donor **38** and D-galactose acceptor **36**, glycosylation of D-galactose donor **42** and octasaccharide **2** furnished a nonasaccharide, which can be transformed to a D-galactoside terminated acceptor (Scheme 5B) (see Supporting Information). A [4 + 9] coupling of nonasaccharide acceptor with both tetrasaccharide donor **38** and **41** failed to produce tridecasaccharide. Decomposition of the donors was detected during these reactions. The reactivity of the C3 hydroxyl group in octasaccharide **2** may be dependent on the steric effect as it reacts with monosaccharide donor **42**, but not pentasaccharide donor **40**. The efficient union of tetrasaccharide **38** and monosaccharide acceptor **36**, and the failure of the glycosylation between tetrasaccharide **38** and nonasaccharide acceptor, indicate that the reactivity of the C3 hydroxyl group in D-galactoside, is influenced by the patterns at the reducing end.

A [(4 + (1 + 3)) + 5] assembly sequence was adopted to tune the reactivity of the C3 hydroxyl group in D-galactoside by placing a trisaccharide at the reducing end (Scheme 5C) (see



Scheme 6. Synthesis of serotype O6 O-antigen tridecasaccharide **1** by [(2 × 1) + (3 + 8)] strategy.

Supporting Information). The condensation of **42** with trisaccharide **43** catalyzed by TMSOTf produced the corresponding tetrasaccharide in 68% yield as β-anomer, before removal of the levulinyl group afforded the corresponding acceptor. Union of donor **41** and the tetrasaccharide acceptor under TMSOTf activation gave the β-linked octasaccharide in 51% yield, indicating that the acceptor activity of 3-OH in D-galactoside bearing tri-heptan unit at the reducing end is suitable for the Le^y tetrasaccharide donor. However, the [8 + 5] coupling of octasaccharide donor and penta-heptan acceptor **31** failed to produce tridecasaccharide, confirming the idea that the final assembly point may not be chosen inside the sterically hindered DD-heptoglycan part. Hence, an evaluation of the reactivity of the C3 hydroxyl group in D-galactose terminated nonasaccharide acceptor was needed. An assembly sequence of [1 + (1 + 8)] was adopted and failed in the glycosylation of the nonasaccharide acceptor and the D-glucosamine donor **44**[40] (Scheme 5D) (see Supporting Information), showing that the octo-heptan fragment reduced the reactivity of the C3 hydroxyl group in D-galactoside too drastically. Octo-heptan **2** should be synthesized initially while the glycosylation at 3-OH of D-galactoside has to be achieved at the D-galactose monosaccharide stage.

Next, a [(2 × 1) + (3 + 8)] assembly sequence based on trisaccharide donor **45** was adopted, where the glycosylation of trisaccharide **45** and octasaccharide **2** was followed by L-fucosylation (Scheme 6). Condensation of donor **46** (see Supporting Information) and acceptor **47**[40] was catalyzed by TMSOTf and produced disaccharide **48** in 53% yield as β-anomer. The glycosylation of **48** with acceptor **36** under TfOH and NIS activation at -20 °C afforded the β-linked trisaccharide **49** in 70% yield. Removal of the TBS by TBAF gave hemiacetal that was transformed to the corresponding *N*-phenyl trifluoroacetimidate **45** in 87% overall yield. A replacement of *N*-

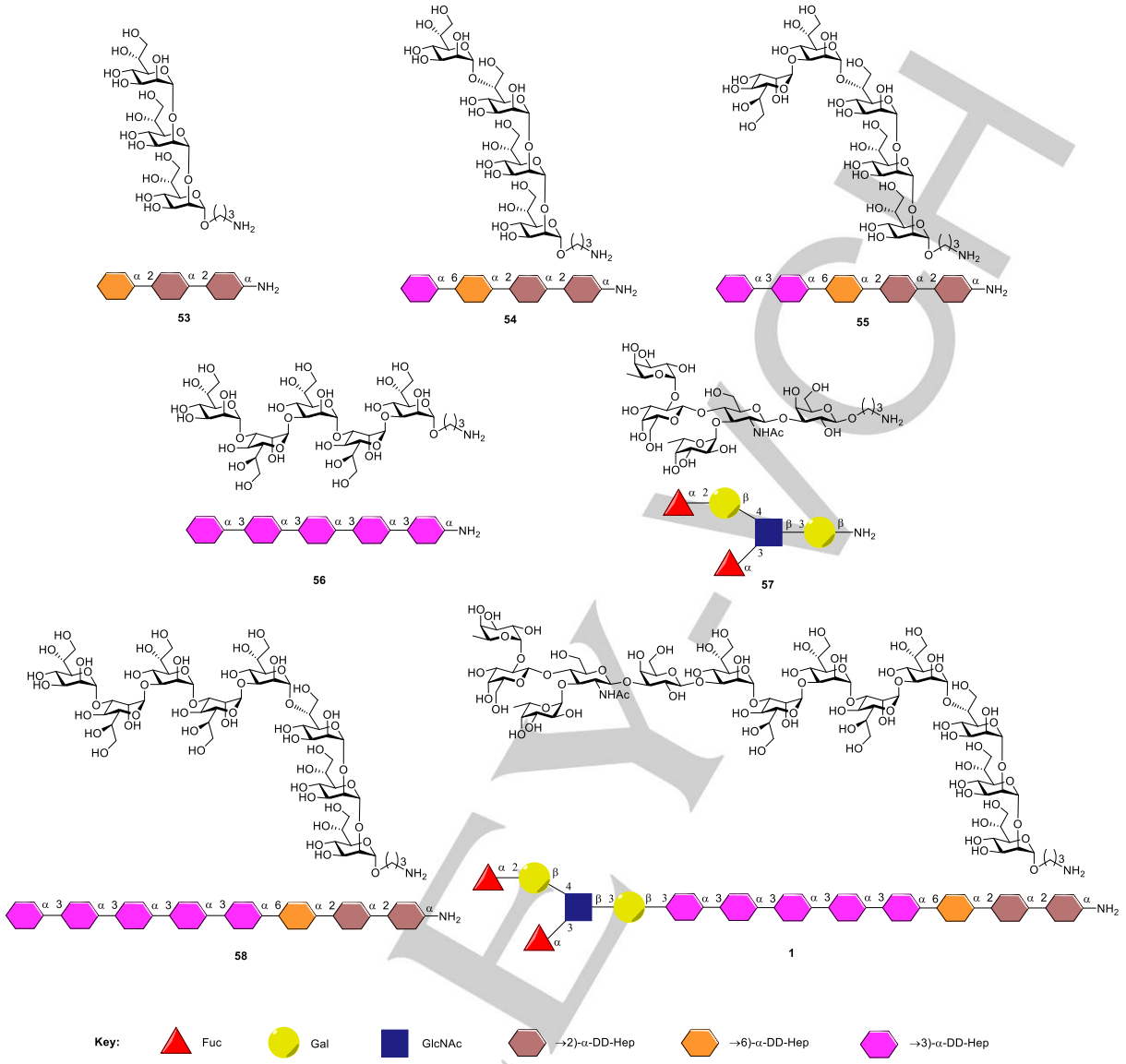


Figure 3. A glycan library related to the *H. pylori* O6 O-antigen.

Troc by *N*-trichloroacetyl (TCA) group avoided the cleavage of amino protecting group in the presence of TBAF. The union of **45** and octasaccharide **2** catalyzed by TMSOTf led to undecasaccharide **50** in 65% yield as β-anomer. Undecasaccharide acceptor **51** was obtained through cleavage of the Lev ester by treatment with hydrazine acetate. A glycosylation reaction between L-fucoside **12** (4 eq) and undecasaccharide acceptor **51** in the presence of TMSOTf gave tridecasaccharide **52** in 52% yield and complete α-selectivity. The failed glycosylation of pentasaccharide donor **40** and octasaccharide acceptor **2**, the success of D-galactose donor **42** and trisaccharide donor **45** to glycosylate **2**, implies that the acceptor reactivity of the octasaccharide **2** may be dependent on the steric effect. On the other hand, the decomposition of L-fucoside-containing donors **40**, **38** and **41** indicated that the failure of [5 + 8] and [4 + (1 + 8)] coupling may be attributed to the lability of the fucosyl linkages during the acid-mediated glycosylation reactions with long-chain glycans. Reduction of the *N*-TCA group in **52** by treatment with Zn-AcOH furnished the corresponding NHAc derivative, while all ester groups were removed by NaOH and NaOMe in a mixture of methanol and tetrahydrofuran. Final deprotection of all benzyl ether and benzyl carbamate groups with Pd/C hydrogenation gave target tridecasaccharide **1** in 82% overall yield. The NMR spectrum of compound **1** is identical with that described in the reference (see Supporting Information).[20]

Finally, based on the Le^y tetrasaccharide unit, DD-heptoglycan unit and different glycosidic linkages, six oligosaccharides (**53**–**58**) related to the *H. pylori* O6 O-antigen were designed and chemically synthesized (Figure 3) (see Supporting Information). Sufficient amounts of pure and well-defined glycans for the generation of glycan microarrays and glycoconjugates were obtained.

Antigenicity Evaluation of Synthetic *H. pylori* O6 O-Antigen. The synthetic oligosaccharides were printed on microarray slides via the reducing end aminopropyl linker (Figure 4) to ass-

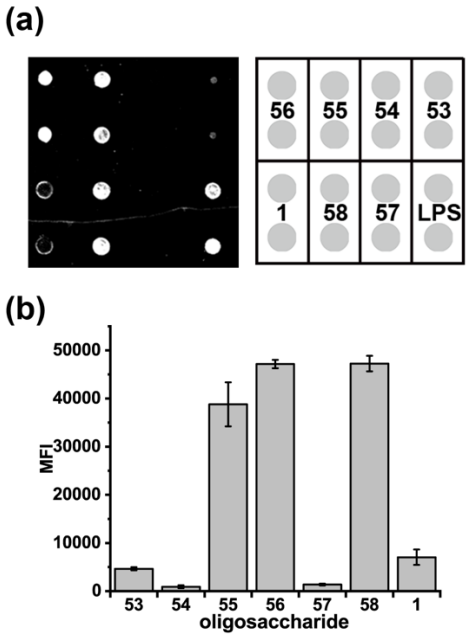


Figure 4. Evaluation of synthetic oligosaccharide fragments by glycan microarray. (a) Representative array scan of *H. pylori* O6 LPS immunized rabbit sera (Left) and printing pattern (Right). (b) Quantification of mean fluorescence intensity (MFI) values of oligosaccharide fragments. Error bars represent SEM of two spots of two separate arrays.

ess the antigenicity of serotype O6 Le^y tetrasaccharide and DD-heptoglycans. Glycan arrays containing all oligosaccharide fragments were screened with the antisera of rabbits after immunization with purified *H. pylori* serotype O6 LPS. The purified serotype O6 LPS was also printed onto the glass slides as positive control.

Among the synthetic oligosaccharides, rabbit IgG antibodies bound specifically to α-(1→3)-linked heptoglycans **55**, **56** and **58** but not to any of the other sequences, including α-(1→2)-, (1→6)-linked heptosides and Le^y tetrasaccharide. Moreover, **1** capped with Le^y tetrasaccharide was not recognized by the rabbit antisera, compared with **58**. The mammalian histo-blood-group antigen Le^y tetrasaccharide in the *H. pylori* O6 O-antigen can't be recognized by antisera but also impede the IgG recognition. Oligosaccharides **56** and **58** that contain more α-(1→3)-linked heptoside motifs bound to rabbit IgG antibodies with a little higher intensity than **55** that contains only one α-(1→3)-linked heptoside motif. The α-(1→3)-linked heptoglycan is a key epitope for designing glycoconjugate vaccines for *H. pylori* serotype O6.

Conclusion

The first total synthesis of *H. pylori* serotype O6 O-antigen tridecasaccharide **1** and related substructures **53**-**58** provided access to pure glycans not available by isolation from natural sources. A trial-and-error approach based on five elaborate assembly sequences was employed and provided an efficient [(2 × 1) + (3 + 8)] assembly of tridecasaccharide **1**. The steric hindrance of DD-heptoglycan, necessitated the final assembly point to be outside the DD-heptoglycan part. The reactivity of the C3 hydroxyl group in D-galactoside, the key assembly point of the tridecasaccharide, was significantly influenced by the functional patterns at the reducing end. Seven oligosaccharides decorated with a propylamine linker laid the basis for immunological investigations into the role of the unique and conserved heptoglycan. Polyclonal rabbit anti-serotype O6 serum from animals immunized with isolated serotype O6 LPS contains antibodies that very specifically bind α-(1→3)-linked heptoglycans **55**, **56** and **58** but not any other sequences. The α-(1→3)-linked heptoglycans are antigenic epitopes while the Le^y tetrasaccharide may rather serve as a decoy to the mammalian immune system, which makes *H. pylori* like a wolf in sheep's clothing[19]. The α-(1→3)-linked heptoglycan is a key motif for designing glycoconjugate vaccines for *H. pylori* serotype O6. Synthetic oligosaccharides are key for the identification of defined cell-surface glycan epitopes as a basis for vaccine development. The challenging synthesis of tridecasaccharide **1** based on trial-and-error approach taught valuable lessons for the syntheses of other sterically hindered long-chain glycans.

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Conflict of interest

The authors declare no conflict of interest.

Keywords: *Helicobacter pylori* • immunology • O-antigen • total synthesis • tridecasaccharide

[1] J. G. Kusters, A. H. M. van Vliet, E. J. Kuipers, *Clin. Microbiol. Rev*. **2006**, *19*, 449-490.
[2] B. J. Marshall, H. M. Windsor, *Med. Clin. North Am.* **2005**, *89*, 313-344.
[3] C. Wang, Y. Yuan, R. H. Hunt, *Am. J. Gastroenterol.* **2007**, *102*, 1789-1798.
[4] A. T. B. Abadi, *Front. Med.* **2016**, *3*, 36.
[5] J. Q. Huang, S. Sridhar, Y. Chen, R. H. Hunt, *Gastroenterology* **1998**, *114*, 1169-1179.
[6] G. D. Eslick, L. L.-Y. Lim, J. E. Byles, H. H.-X. Xia, N. J. Talley, *Am. J. Gastroenterol.* **1999**, *94*, 2373-2379.
[7] *Helicobacter* and Cancer Collaborative Group, *Gut* **2001**, *49*, 347-353.
[8] F. Kamangar, S. M. Dawsey, M. J. Blaser, G. I. Perez-Perez, P. Pietinen, C. J. Newschaffer, C. C. Abnet, D. Albanes, J. Virtamo, P. R. Taylor, *J. Natl. Cancer Inst.* **2006**, *98*, 1445-1452.
[9] International Agency for Research on Cancer Working Group on the Evaluation of Carcinogenic Risks to Humans, Lyon: IARC. **1994**, *61*, 177-240.
[10] S. M. Campo, A. Zullo, C. Hassan, S. Morini, *Recent Pat. Antiinfect Drug Discov*. **2007**, *2*, 11-17.
[11] V. De Francesco, F. Giorgio, C. Hassan, G. Manes, L. Vannella, C. Panella, E. Ierardi, A. Zullo, *J. Gastrointest. Liver* **2010**, *19*, 409-414.
[12] G. Ayala, W. I. Escobedo-Hinojosa, C. F. de la Cruz-Herrera, I. Romero, *World J. Gastroenterol.* **2014**, *20*, 1450-1469.
[13] M. Selgrad, P. Malfertheiner, *Curr. Opin. Pharmacol.* **2008**, *8*, 593-597.

[14] M. Zeng, X.-H. Mao, J.-X. Li, W.-D. Tong, B. Wang, Y.-J. Zhang, G. Guo, Z.-J. Zhao, L. Li, D.-L. Wu, D.-S. Lu, Z.-M. Tan, H.-Y. Liang, C. Wu, D.-H. Li, P. Luo, H. Zeng, W.-J. Zhang, J.-Y. Zhang, B.-T. Guo, F.-C. Zhu, Q.-M. Zou, *Lancet* **2015**, *386*, 1457-1464.

[15] D. Esmaeilli, A. M. Mobarez, A. H. Salmanian, A. Z. Hosseini, *Iran. J. Microbiol.* **2013**, *5*, 142-146.

[16] M. A. Monteiro, S. Britton, L. A. Applebee, S. Baqar, *Vaccine* **2011**, *29*, 3098-3102.

[17] J. A. Ferreira, L. Silva, M. A. Monteiro, M. A. Coimbra, *Carbohydr. Chem.* **2012**, *37*, 160-193.

[18] M. A. Monteiro, F. S. Michael, D. A. Rasko, D. E. Taylor, J. W. Conlan, K. H. Chan, S. M. Logan, B. J. Appelmelk, M. B. Perry, *Biochem. Cell Biol.* **2001**, *79*, 449-459.

[19] M. A. Monteiro, *Carbohydr. Chem. Biochem.* **2001**, *57*, 99-158.

[20] G. O. Aspinall, M. A. Monteiro, R. T. Shaver, L. A. Kurjanczyk, J. L. Penner, *Eur. J. Biochem.* **1997**, *248*, 592-601.

[21] G. O. Aspinall, M. A. Monteiro, *Biochemistry* **1996**, *35*, 2498-2504.

[22] a) M. Sűsskind, L. Brade, H. Brade, O. Holst, *J. Biol. Chem.*, **1998**, *273*, 7006-7017. b) M. K. Gurjar, A. Talukdar, *Tetrahedron* **2004**, *60*, 3267-3271.

[23] S. Yokota, K. Amano, S. Hayashi, T. Kubota, N. Fujii, T. Yokochi, *Infect. Immun*. **1998**, *66*, 3006-3011.

[24] S. Yokota, K. Amano, Y. Shibata, M. Nakajima, M. Suzuki, S. Hayashi, T. Kubota, N. Fujii, T. Yokochi, *Infect. Immun*. **2000**, *68*, 151-159.

[25] P. H. Seeberger, D. B. Werz, *Nature* **2007**, *446*, 1046-1051.

[26] a) P. Wang, C.-X Huo, S. Lang, K. Caution, S. T. Nick, P. Dubey, R. Deora, X. Huang, *Angew. Chem. Int. Ed.* **2020**, *59*, 6451-6458; *Angew. Chem.* **2020**, *132*, 6513-6520. b) L. Liu, J. Zha, A. DiGiandomenico, D. McAllister, C. K. Stover, Q. Wang, G-J. Boons, *Angew. Chem. Int. Ed.* **2015**, *54*, 10953-10957; *Angew. Chem.* **2015**, *127*, 11103-11107. c) C. L. Pereira, A. Geissner, C. Anish, P. H. Seeberger, *Angew. Chem. Int. Ed.* **2017**, *56*, 13973-13978; *Angew. Chem.* **2017**,*129*, 14161-14166. d) K.-C. Chu, C.-Y. Wu, *Future Med. Chem.* **2012**, *4*, 1767-1770.

[27] a) R. U. Lemieux, H. Driguez, *J. Am. Chem. Soc.* **1975**, *97*, 4063-4069. b) U. Spohr, R. U. Lemieux, *Carbohydr. Res.* **1988**, *174*, 211-237. c) T. Buskas, Y.-H. Li, G.-J. Boons, *Chem. Eur. J.* **2005**, *11*, 5457-5467. d) T. Zhu, G.-J. Boons, *Chem. Eur. J.* **2001**, *7*, 2382-2389. e) M. K. Spassova, W. G. Bornmann, G. Ragupathi, G. Sukenick, P. O. Livingston, S. J. Danishefsky, *J. Org. Chem.* **2005**, *70*, 3383-3395. f) S. J. Danishefsky, V. Behar, J. T. Randolph, K. O. Lloyd, *J. Am. Chem. Soc.* **1995**, *117*, 5701-5711. g) K.-K. T. Mong, C.-H. Wong, *Angew. Chem. Int. Ed.,* **2002**, *41*, 4087-4090; *Angew. Chem.* **2002**, *114*, 4261-4264.

[28] a) H. Yu, Y.-H. Li, Z.-G. Wu, L. Li, J. Zeng, C. Zhao, Y.-J. Wu, N. Tasnima, J. Wang, H.-D. Liu, M. R. Gadi, W.-Y. Guan, P. G. Wang, X. Chen, *Chem. Commun.* **2017**, *53*, 11012-11015. b) H. Yu, X. Chen, *Org. Biomol. Chem.* **2016**, *14*, 2809-2818. c) P. M. Danby, S. G. Withers, *ACS Chem. Biol.* **2016***, 11,* 1784-1794.

[29] a) C.-H. Hsu, S.-C. Hung, C.-Y. Wu, C.-H. Wong, *Angew. Chem. Int. Ed.* **2011**, *50*, 11872-11923; *Angew. Chem.* **2011**, *123*, 12076-12129. b) C. M. Pedersen, L. G. Marinescu, M. Bols, *Chem. Commun.* **2008**, *21*, 2465-2467.

[30] a) S. van der Vorm, T. Hansen, J. M. A. van Hengst, H. S. Overkleeft, G. A. van der Marel, J. D. C. Codée, *Chem. Soc. Rev.* **2019**, *48*, 4688-4706. b) A. Behera, D. Rai, S. S. Kulkarni, *J. Am. Chem. Soc.* **2020**, *142*, 456-467. c) C.-J. Qin, B. Schumann, X.-P. Zou, C. L. Pereira, G.-Z. Tian, J. Hu, P. H. Seeberger, J. Yin, *J. Am. Chem. Soc.* **2018**, *140*, 3120-3127.

[31] a) R. R. Schmidt, J. Michel, *Angew. Chem. Int. Ed.* **1980**, *19*, 731-732; *Angew. Chem.* **1980**, *92*, 763-764. b) R. J. Ferrier, R. W. Hay, N. Vethaviyasar, *Carbohydr. Res.* **1973**, *27*, 55-61. c) Y. Li, Y. Yang, B. Yu, *Tetrahedron Lett.* **2008**, *49*, 3604-3608. d) B. Yu, *Acc. Chem. Res.* **2018**, *51*, 507-516. e) M. M. Nielsen, C. M. Pedersen, *Chem. Rev.* **2018**, *118*, 8285-8358. f) G. Báti, J.-X. He, K. B. Pal, X.-W. Liu, *Chem. Soc. Rev.* **2019**, *48*, 4006-4018.

[32] a) X. Geng, V. Y. Dudkin, M. Mandal, S. J. Danishefsky, *Angew. Chem. Int. Ed.* **2004**, *43*, 2562-2565; *Angew. Chem.* **2004***, 116*, 2616-2620. b) P. Nagorny, B. Fasching, X.-C. Li, G. Chen, B. Aussedat, S. J. Danishefsky, *J. Am. Chem. Soc.* **2009**, *131*, 5792-5799.

[33] a) Y. Wu, D.-C. Xiong, S.-C. Chen, Y.-S. Wang, X.-S. Ye, *Nat. Commun.* **2017**, *8*, 14851-14857. b) N. Trattnig, M. Blaukopf, J.-F. Bruxelle, R. Pantophlet, P. Kosma, *J. Am. Chem. Soc.* **2019**, *141*, 7946-7954. c) V. Pozsgay, J. Kubler-Kielb, B. Coxon, P. Santacroce, J. B. Robbins, R. Schneerson, *J. Org. Chem.* **2012**, *77*, 5922-5941. d) L.-Z. Wang, S.-J. Feng, L. An, G.-F. Gu, Z.-W. Guo, *J. Org. Chem.* **2015**, *80*, 10060-10075.

[34] a) X.-P. Zou, C.-J. Qin, C. L. Pereira, G.-Z. Tian, J. Hu, P. H. Seeberger, J. Yin, *Chem. Eur. J.* **2018**, *24*, 2868-2872. b) G.-Z. Tian, C.-J. Qin, Z.-H. Liu, D.-C. Shen, X.-P. Zou, J.-J. Fu, J. Hu, P. H. Seeberger, J. Yin, *Chem. Commun*. **2020**, *56*, 344-347.

[35] a) C.-H. Liang, S.-K. Wang, C.-W. Lin, C.-C. Wang, C.-H. Wong, C.-Y. Wu, *Angew. Chem. Int. Ed.* **2011**, *50*, 1608-1612; *Angew. Chem.* **2011**, *123*, 1646-1650. b) U. Westerlind, H. Schröder, A. Hobel, N. Gaidzik, A. Kaiser, C. M. Niemeyer, E. Schmitt, H. Waldmann, H. Kunz, *Angew. Chem. Int. Ed.* **2009**, *48*, 8263-8267; *Angew. Chem.* **2009***, 121*, 8413-8417. c) C. L. Pereira, A. Geissner, C. Anish, P. H. Seeberger, *Angew. Chem. Int. Ed.* **2015**, *54*, 10016-10019; *Angew. Chem.* **2015**, *127*, 10154-10157. d) A. Reinhardt, Y. Yang, H. Claus, C. L. Pereira, A. D. Cox, U. Vogel, C. Anish, P. H. Seeberger, *Chemistry & Biology* **2015**, *22*, 38-49. e) C. E. Martin, F. Broecker, S. Eller, M. A. Oberli, C. Anish, C. L. Pereira, P. H. Seeberger, *Chem. Commun.* **2013**, *49*, 7159-7161. f) C. Anish, C. E. Martin, A. Wahlbrink, C. Bogdan, P. Ntais, M. Antoniou, P. H. Seeberger, *ACS Chem. Biol.* **2013**, *8*, 2412-2422. g) C. E. Martin,; F. Broecker, M. A. Oberli, J. Komor, J. Mattner, C. Anish, P. H. Seeberger, *J. Am. Chem. Soc.* **2013**, *135*, 9713-9722. h) C. Anish, X. Guo, A. Wahlbrink, P. H. Seeberger, *Angew. Chem. Int. Ed.* **2013**, *52*, 9524-9528; *Angew. Chem.* **2013***, 125,* 9702-9706.

[36] J. D. M. Olsson, S. Oscarson, *Carbohydr. Res.* **2010**, *345*, 1331-1338.

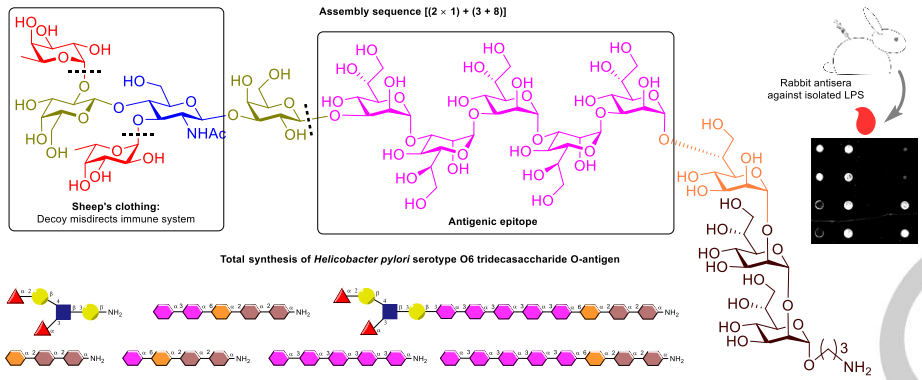
[37] M. Adinolfi, A. Iadonisi, A, Ravidà, M. Schiattarella, *Synlett.* **2004**, *2*, 275-278.

[38] a) H. Yu, B. Yu, X.-Y. Wu, Y.-Z. Hui, X.-W. Han, *J. Chem. Soc., Perkin Trans. 1*, **2000**, *9*, 1445-1453. b) A.-R. de Jong, B. Hagen, V. van der Ark, H. S. Overkleeft, J. D. C. Codée, G. A. Van der Marel, *J. Org. Chem*. **2012**, *77*, 108-125.

[39] A. R. Podilapu, S. S. Kulkarni, *Org. Lett.* **2014**, *16*, 4336-4339.

[40] J. Kandasamy, F. Schuhmacher, H. S. Hahm, J. C. Klein, P. H. Seeberger, *Chem. Commun.* **2014**, *50*, 1875-1877.

Entry for the Table of Contents



The *Helicobacter pylori* serotype O6 tridecasaccharide O-antigen containing a terminal Le^y tetrasaccharide and a unique DD-heptoglycan was chemically synthesized for the first time. Immunological investigation based on seven synthetic oligosaccharides revealed that the α-(1→3)-linked DD-heptoglycan is a key motif for designing glycoconjugate vaccines against *H. pylori*, while the Le^y tetrasaccharide may rather serve as a decoy to the immune system.