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Synthesis of a conjugable hexasaccharide corresponding to the capsular polysaccharide of *Campylobacter jejuni* strain BH0142

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ABSTRACT

The first assembly of a conjugation-ready hexasaccharide from the capsular glycan of *C. jejuni.* strain BH0142 has been accomplished. The synthesis features the efficient preparation of 6-deoxy-D-*ido*-heptopyranosyl fluoride donors proceeding from allyl α-D-*C*-glucopyranoside by a C1-to-C5 switch strategy with radical dehydroxymethylative fluorination as a key step, stereocontrolled construction of 1,2-*trans*-α-D-*ido*-heptopyranosidic bonds and of 1,2-*cis*-α-D-galactopyranosidic linkages. The obtained target oligosaccharide sets a solid foundation for making structurally-defined multivalent glycoconjugate vaccine candidates against *C. jejuni.* infections.

Campylobacter jejuni (*C. jejuni*) is one of the leading causes of human gastroenteritis worldwide [1]. Although campylobacteriosis is both preventable and treatable in most cases, it still poses enormous challenges to animal and public health. *C. jejuni* infections can result in serious sequelae such as a rare autoimmune disease known as Guillain–Barré Syndrome [2] and reactive arthritis [3]. It is estimated that *C. jejuni* is linked to 40% of all new cases of Guillain–Barré Syndrome [4]. *Campylobacter* infections are associated with the death of around 530,000 children every year [5]. Globally, resistance to clinical antibiotics against *C. jejuni* infections is increasingly rising and therefore creates the urgent need for alternative therapeutics.

Glycoconjugate vaccines derived from native and synthetic glycans have been recognized as efficient tools to combat pathogenic infections and to stem antibiotic resistance crisis [6,7]. A preeminent example in point is the approved semi-synthetic vaccine against *Haemophilus influenzae* Type B [8]. The *C. jejuni* capsular polysaccharides (CPSs) are the key antigenic determinants of the Penner serotyping scheme [9] and represent potential vaccine targets [10,11]. Natural *C. jejuni* CPS-based glycoconjugate vaccines showed significant immunogenicity in animal test [12], highlighting the potential of such conjugates in discovery and development of new therapeutics to combat *C. jejuni* infections. These advantageous properties might be linked to unique structures of *C. jejuni* CPSs, which are commonly embedded by uncommon D/L-*glycero*-D/L-heptosyl units or related 6-deoxy derivatives [13]. Among them, the glycans containing 6-deoxy-D-*ido*-heptopyranosyl (6d*ido*Hep*p*) units and its L-*glycero* congeners caught our attention because these constructs have not been found in other organisms.

Considerable efforts have been devoted to development of glycoconjugate vaccines for the prevention of campylobacteriosis in the past 20 years, however, there are no approved vaccines available [10,14]. We recently established a novel C1-to-C5 switch strategy for the synthesis of uncommon D/L-6-deoxy-heptopyranosyl fluorides from the easily accessible allyl α-C-hexopyranosides relying on oxidative radical decarboxylative fluorination of uronic acids [15]. The transformation has been applied in the first assembly of a hexasaccharide related to the *C. jejuni.* strain CG8486 capsular polysaccharide (Scheme 1A) consisting of $\rightarrow$3)-β-D-6d*ido*Hep*p*-(1$\rightarrow$4)-β-D-Glc*p*NAc-(1$\rightarrow$. The synthesis features the preparation of 6d*gul*Hep*p* fluoride from allyl α-D-*C*-galactoside and the indirect construction of the challenging 1,2-*cis*-β-D-idopyranosides by epimerizing the C2 configuration of 1,2-*trans*-β-D-gulopyranosides [15,16].

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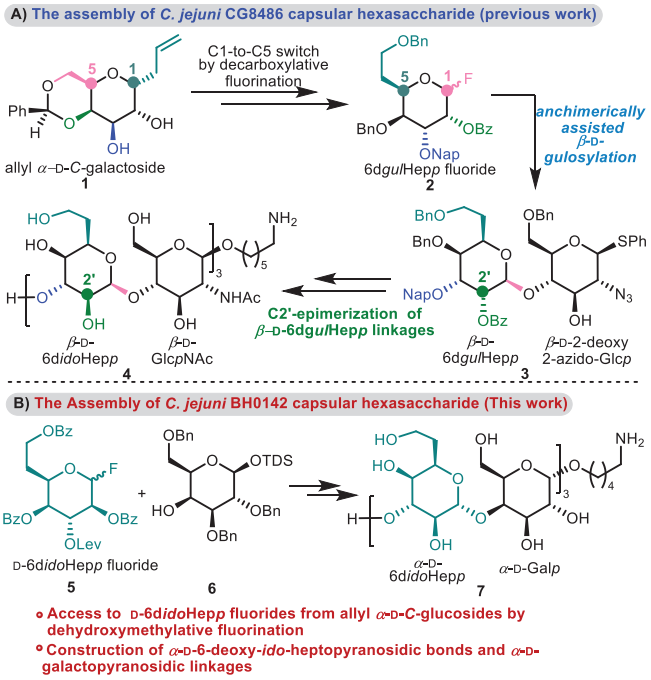
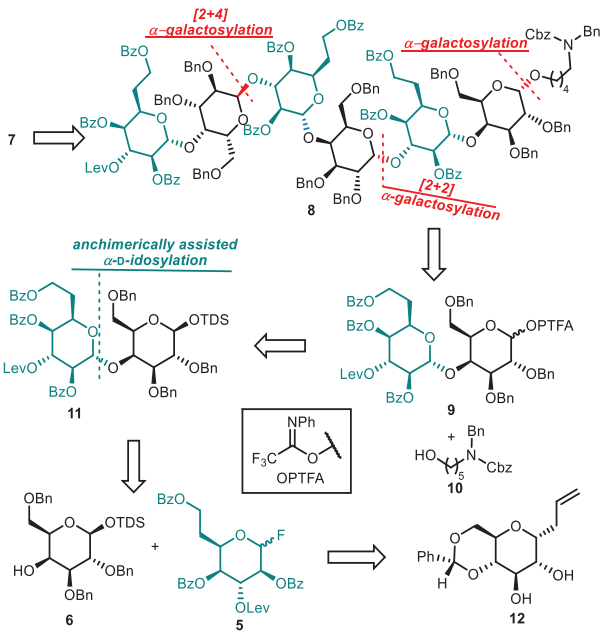


Scheme 1. Synthesis of *C. jejuni* strain CG8486 and BH0142 capsular hexasaccharides.



Scheme 2. Retrosynthetic analysis for the capsular hexasaccharide **7**.

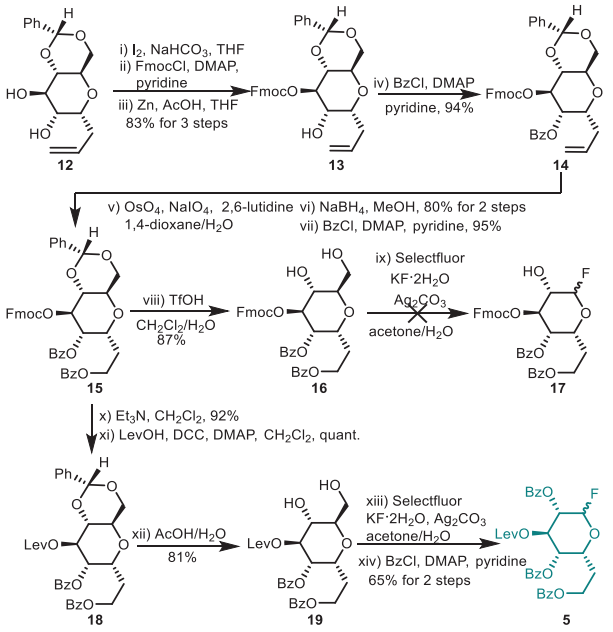
Given that there are 35 types of *C. jejuni* CPSs recognized so far, it is desirable to make multivalent glycoconjugate vaccines based on different glycans to prevent pathogenesis of *C. jejuni* [17]. As part of our efforts to culminate in synthesis and biological evaluation of structurally defined multivalent synthetic vaccines against *C. jejuni* infections, we herein describe blockwise synthesis of a conjugable hexasaccharide **7** corresponding to the *C. jejuni.* strain BH0142 capsular polysaccharide composed of →3)-α-D-6d*ido*Hepp-(1→4)-α-D-Gal*p*-(1→ (Scheme 1B). This assembly is characterized by the convenient preparation of unique D-6d*ido*Hep*p* fluoride from the readily available allyl α-D-*C*-glucoside through oxidative radical dehydroxymethylative fluorination, the convenient construction of 1,2-*trans*-α-D-*ido*-heptopyranosidic bonds relying on the catalytic activation of orthogonally protected idopyranosyl fluoride, and of the stereoselective formation of 1,2-*cis*-α-D-galactopyranosidic linkages.

Structurally, target molecule **7** is a trimer derivative of disaccharide repeating unit. Accordingly, we chose to take a convergent approach using a {2 + [2 + 2]} strategy to reach the goal. As shown in Scheme 2, we attempted to generate **7** by unmasking the fully protected hexasaccharide **8**. The assembly of **8** could be achieved by iterative employment of the key disaccharide *N*-phenyl trifluoroacetimidate (PTFA) **9** as glycosylating agent through stereoselective formation of 1,2-*cis*-α-D-galactopyranosidic linkages with aglycone **10** [18] and the C3-OH of idosyl moiety. Disaccharide donor **9** could be traced back to **11** bearing a temporary protecting group dimethylthexylsilyl (TDS) at the reducing end. The preparation of **11** entailed stereocontrolled construction of 1,2-*trans*-α-D-glycosidic bonds between 6d*ido*Hep*p* donor with galactosyl acceptor.

Inspired by the work of Pakulski who successfully applied benzoyl (Bz)-protected idopyranosyl donor in synthesis of α-D-idosides [19], we envisaged that the desired disaccharide **11** could be achieved by means of the anchimerically assisted glycosylation with 6-deoxy-D-*ido*-heptopyranosyl donor bearing C2-, C4- and C7-benzoates. In addition, in order to extend the sugar chain at C3 position of idosyl moiety, an orthogonal and temporary protecting group should be installed at that position. On the other hand, the construction of α-D-galactopyranosidic linkage required the incorporation of C2-ether type substituent. Furthermore, to increase the nucleophilicity of the axial C4-OH of Gal*p* units favoring glycosylation at that sterically hindered position, the masking of the hydroxy groups adjacent to that group with ether-type protecting group should be preferred. With those considerations in mind and inspired by synthesis of rare glycosyl fluorides by use of radical dehydroxymethylative fluorination [20,21] as well as their catalytic activation [22] to engage in glycosylation, we would like to use 6d*ido*Hep*p* fluoride **5** as glycosyl donor to introduce the 6d*ido*Hep*p* residue and the literature-known compound **6** [23] as the galactosyl building block that is protected by benzyl (Bn) groups at C2-, C3- and C6-OHs. Fluoride **5** could be prepared proceeding from allyl α-*C*-glucopyranoside **12** [15] by a C1-to-C5 switch strategy through oxidative radical dehydroxymethylative fluorination as a key transformation.

Our synthesis commenced with the preparation of D-6d*ido*Hep*p* fluoride **5** (Scheme 3). We initially attempted to use 9-fluorenylmethyloxy carbonyl (Fmoc) to mask the C3-OH of 6d*ido*Hep*p* moiety because of the reaction conditions for its installation and deprotection leaving benzoates intact, and of its tolerance of both Zn/AcOH- and $NaBH_4$-mediated reduction reaction (*vide infra*). To this end, diol **12** was subjected to intramolecular iodoetherification [24], the ensuing reaction with FmocCl, and Zn/AcOH-promoted reductive ring opening [25] of iodomethyl tetrahydrofuran. The reaction sequence delivered the desired product **13** in 83% yield over three steps with the liberation of the C2-OH and the recovery of the anomeric allyl substituent. At this stage, benzoylation of **13** was uneventfully achieved with BzCl in the presence of 0.01 equiv. of 4-*N*,*N*-dimethylpyridine (DMAP) in pyridine. The reaction afforded the expected benzoate **14** in 94% yield with Fmoc group intact. It is important to note that the larger amounts of DMAP caused the removal of the Fmoc group, producing thus 2,3-di-*O*-benzoate **S2** (see Supporting information).

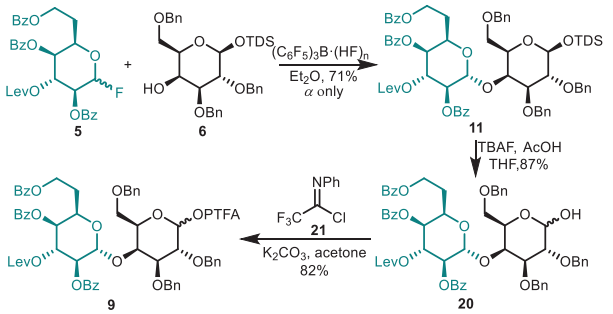
Oxidative cleavage of the olefin using OsO_4/$NaIO_4$ in aqueous dioxane, subsequent reduction of the resulting aldehyde using $NaBH_4$, and benzoylation converted **14** into benzoate **15**



Scheme 3. Preparation of D-6d*ido*Hep*p* fluoride **5**.

in 76% yield. The removal of the benzylidene in **15** using trifluoromethanesulfonic acid (TfOH)-catalyzed transacetalization in MeOH afforded diol **16** in 87% yield. Exposure of **16** to Ag_2CO_3-induced radical dehydroxymethylative fluorination reaction [20,21], however, resulted in a mess reaction. We assumed that this unrewarding result might be attributed to lability of Fmoc group to the used conditions. To address this issue, we resorted to levulinoyl (Lev) to protect the C3-OH of 6d*ido*Hep*p* unit because of its tolerance of radical dehydroxymethylative fluorination reaction. Levulinate **18** was achieved by the cleavage of Fmoc with Et_3N in CH_2Cl_2 and the subsequent introduction of Lev using dicyclohexylcarbodiimide (DCC)-mediated condensation with levulinic acid [26]. Chemoselective radical dehydroxymethylative fluorination of the primary hydroxy group over the secondary one in diol **19**, prepared by deprotecting the benzylidene in **18**, afforded the desired glycosyl fluoride as an anomeric mixture of α/β 2/1. At this point, the required D-6d*ido*Hep*p* fluoride **5** was prepared in 29% overall yield involving a 12-step reaction sequence from allyl α-D-C-glucoside **12**.

With fluoride **5** in hand, we next focused on glycosylation of **6** [23] with **5** to generate the requisite disaccharide imidate **9** (Scheme 4). We recently found that 0.1 equiv. of $(C_6F_5)_3B\cdot(HF)_n$ as catalyst enable glycosylation of various alcohol acceptors with disarmed glycosyl fluorides [22]. To our delight, the $(C_6F_5)_3B\cdot(HF)_n$-

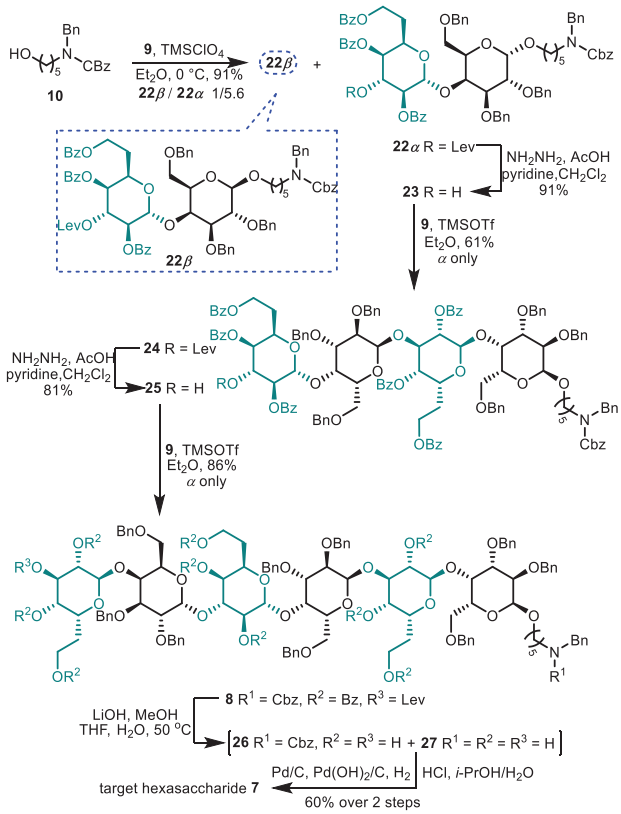


Scheme 4. Preparation of disaccharide *N*-phenyltrifluoroacetimidate **9**.

catalyzed coupling reaction of **5** with **6** smoothly and stereoselectively afforded the desired disaccharide **11** in 71% yield due to neighboring group participation of the C2-benzoate in **5**. The anomeric $^1J_{C\text{-}H}$ 174 Hz of the idosyl unit is diagnostic of 1,2-*trans*-α-D-configuration of the newly formed glycosidic bond. In addition, the silyl group TDS was found to survive the glycosylation conditions. Upon treatment with acetic acid-buffered tetrabutylammonium fluoride (TBAF) followed by reaction of the resulting hemiacetal with *N*-phenyltrifluoroacetiminoyl chloride **21** in the presence of K_2CO_3 in acetone, compound **11** was transformed into glycosyl *N*-phenyltrifluoroacetimidate donor **9** in 71% isolated yield over two steps.

With disaccharide donor **9** secured, we moved our attention to the equipment of the capping linkage at the reducing end by constructing 1,2-*cis*-α-D-galactopyranosidic linkages, the formation of which is not trivial, especially for relatively reactive primary alcohols. Various tactics are adopted to address this issue including employment of the anomeric effects [27], judicious choice of the protecting groups [28], recourse to the solvent effects [29]. As such, we evaluated the effects of solvents, catalysts, and reaction temperatures on the outcome of the coupling between **9** and **10**. As compiled in Table S1 (Supporting information), the results revealed that trimethylsilyl perchlorate ($TMSClO_4$)-catalyzed glycosylation of **9** with **10** in ether furnished glycosides **22*α*** and **22*β*** in the highest 5.6/1 stereoselectivity and 91% total yield, favoring the α-isomer as the expected product. We attributed these observations to the cooperation of the solvent effects of ether and the utility of perchlorate counterions of the catalyst. Both of them have proven to favor the formation of the axial-type glycosidic bonds [30,31].

With disaccharides **9** and **22*α*** in hand, we set out to conduct the sugar chain assembly. As depicted in Scheme 5, the orthogonal cleavage of the levulinate using hydrazine acetate in **22*α*** proceeded well to provide **23** in an excellent yield of 91%, ready



Scheme 5. Synthesis of target hexassacharide **7**.

for glycosylation at its C3′-OH. Trimethylsilyl trifluoromethanesulfonate (TMSOTf)-catalyzed coupling of **23** with **9** in ether gave rise to tetrasaccharide **24** in 61% yield with exclusive α-stereoselectivity. Compared to glycosylation of **10**, this improved stereochemical outcome might be associated with the weaker nucleophilicity of the axially-oriented secondary C3′-OH in **23** than the primary hydroxy group of **10**, which shift the glycosylation reaction toward a S_N1-like process [32]. The same manipulations as those of **24** involving the cleavage of levulinate and glycosylation with **9**, tetrasaccharide **25** was converted into hexasaccharide **8** in 86% yield.

Next, deprotection of **8** *en route* to target glycan **7** was attempted. For this end, we exposed **8** to LiOH-promoted hydrolysis of esters in THF/H_2O at 50 °C. After stirring overnight, the mass spectrometric analysis of the crude reaction solution showed that the reaction resulted in a mixture of **26** with complete cleavage of the benzoates and the levulinate as well as **27** with additional deprotection of the benzyloxy carbamate (Cbz). Due to difficulty in separating **27** from **26** by silica gel column chromatography, the mixture was treated with $Pd(OH)_2$/C and Pd/C under hydrogen atmosphere in *i*-PrOH/H_2O at room temperature. The hydrogenolysis reaction proceeded smoothly and provided target molecule **7** in 60% yield over two steps. The structure of **7** was corroborated by the extensive 1D and 2D NMR experiments (Supporting information), and the configuration of the constructed glycosidic bonds was verified by the corresponding anomeric $^1J_{C\text{-}H}$ coupling constants (for α-D-6d*ido*Hep*p* bonds, $^1J_{C1\text{-}H1} = 167.6$ and 168.2 (overlapped) Hz; for α-D-Gal*p* bonds, $^1J_{C1\text{-}H1} = 171.2$ (overlapped) and 171.3 Hz).

In conclusion, we have accomplished the first assembly of a hexasaccharide corresponding to the capsular polysaccharide of *C. jejuni* strain BH0142. The synthesis features the expeditious preparation of the orthogonally protected 6d*ido*Hep*p* fluoride and $(C_6F_5)_3B\cdot(HF)_n$-catalyzed glycosylation of that donor, leading to efficient construction of the 1,2-*trans*-α-D-*ido*-heptopyransidic bonds through anchimeric assistance. In addition, 1,2-*cis*-α-D-galactopyranosidic linkages are highly stereoselectively achieved by employing the solvent effects of ether together with the capability of perchlorate counterions to favor α-stereoselectivity of glycosylation reaction. The accessibility of oligosaccharide **7** sets a solid foundation for glycoconjugate vaccine development. The established strategy for the construction of glycosidic bonds would facilitate synthesis of related glycans arising from *C. jejuni* capsular polysaccharides.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.cclet.2022.107804.

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