Available online at www.sciencedirect.com
ScienceDirect
Carbohydrate Research 343 (2008) 1034–1040
Carbohydrate RESEARCH

# The chemical structure and genetic locus of *Campylobacter jejuni* CG8486 (serotype HS:4) capsular polysaccharide: the identification of 6-deoxy-D-*ido*-heptopyranose

Yu-Han Chen,[a] Frédéric Poly,[b] Zbigniew Pakulski,[c] Patricia Guerry[b,*] and Mario A. Monteiro[a,*]

[a]*Department of Chemistry, University of Guelph, Guelph, ON, Canada N1G 2W1*
[b]*Enteric Diseases Department, Naval Medical Research Center, Silver Spring, MD 20910, USA*
[c]*Institute of Organic Chemistry, Polish Academy of Sciences, Kasprzaka 44, PL-01224 Warsaw, Poland*

Received 21 January 2008; received in revised form 25 February 2008; accepted 27 February 2008
Available online 4 March 2008

**Abstract**—In line with our on-going efforts to create a multivalent anti-*Campylobacter jejuni* vaccine based on its capsule polysaccharides (CPSs), we report here the chemical structure and the genetic locus of the CPS produced by *C. jejuni* strain CG8486, which belongs to the serotype HS:4 CPS complex. *C. jejuni* CG8486 CPS was observed to be composed of approximately 17 disaccharide repeating blocks of 4-substituted *N*-acetyl-β-D-glucopyranosamine and 3-substituted 6-deoxy-β-D-*ido*-heptopyranose. A small number of 6-deoxy-β-D-*ido*-heptopyranose units were observed to carry *O*-methyl phosphoramidate moieties at the O-2 or O-7 position. The gene content and organization of the CPS locus of *C. jejuni* CG8486 were comparable to those of *C. jejuni* strains NCTC 11168 and 81-176, but several CG8486 CPS genes were observed to be more divergent from those present in the CPS loci of NCTC 11168 and 81-176 CPS, which indicated that there are genetic characteristics specific to the *C. jejuni* HS:4 CPS complex. The efficacy of a glycoconjugate vaccine based on *C. jejuni* CG8486 CPS is presently being tested in an animal model, the results of which will be presented in future communications.


*Keywords:* *Campylobacter jejuni*; Capsule polysaccharide; Genetic locus; 6-Deoxy-*ido*-heptopyranose; Structural characterization

## 1. Introduction

The majority of bacterial gastroenteritis in humans is caused by *Campylobacter jejuni*.[1] This Gram-negative bacterium has been shown to produce lipo-oligosaccharide (LOS) and capsule polysaccharide (CPS) as cell-surface carbohydrate components,[2] of which the most striking structural features are the expression of sialylated gangliosides by the LOSs,[3,4] and of heptose units (commonly present as 6-deoxy-heptoses) of unusual configuration and *O*-methyl phosphoramidate (MeOPN) moieties by the CPS.[5–9] A recent study has also identified the lipid anchor of *C. jejuni* CPS (strain 81-176) as a dipalmitoyl-glycerophosphate, with two residues of ester-linked hexadecanoic acids, and in which the CPS is linked to the anchor through a phosphate group.[10] Other Campylobacter species such as *C. coli*[11] and *C. lari*[12,13] also carry CPSs with 6-deoxy-heptose components, but no ganglioside antigens have yet been observed in their LOSs.[12–14] Due to the structural similarity between *C. jejuni* LOS and ganglioside glycoforms present in the human brain, in some cases, infection by *C. jejuni* may cause Guillain–Barré or Miller–Fisher neurological syndromes due to the production of anti-ganglioside antibodies.[15] In addition, *C. jejuni*

*Abbreviations*: GC, gas chromatography; MS, mass spectrometry; NMR, nuclear magnetic resonance; CPS, capsule polysaccharide

* Corresponding authors. Tel.: +1 519 824 4120x53447; fax: +1 519 766 1499 (M.A.M.); tel.: +1 301 319 7662; fax: +1 301 319 7679 (P.G.); e-mail addresses: patricia.guerry@med.navy.mil; monteiro@uoguelph.ca

0008-6215/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carres.2008.02.024

LOSs and CPSs have also been reported to be involved in adherence, serum resistance, and in invasion of epithelial cells.[16,17]

The fact that serum antibodies are raised against the CPSs of *C. jejuni* suggests that these antigens are readily available for detection by the immune system and thus may have the potential to be used as immunogens in an anti-*C. jejuni* vaccine. During the past three years, we have synthesized and immunologically evaluated the first glycoconjugate vaccine against *C. jejuni*.[18] The glycoconjugate vaccine, formulated using the structurally defined[8] CPS of *C. jejuni* strain 81-176 (H:23/HS:36 CPS serotype complex) and the carrier protein $CRM_{197}$ (genetically modified diphtheria toxin), showed that it could trigger elevated and sustainable levels of IgG $CPS_{81\text{-}176}$-specific antibodies, and that it was protective in a mouse model of infection.[18] Now, our goal is to synthesize additional glycoconjugate preparations composed of *C. jejuni* CPSs belonging to other prevalent serotypes to yield a multivalent glycoconjugate vaccine.

To this end, we describe here the structural characterization and the analysis of the genetic locus of a *C. jejuni* CPS assignable to the HS:4 serotype complex (strain CG8486), which is responsible for a high number of *C. jejuni* infections around the world. *C. jejuni* strain CG8486 was isolated from a patient with inflammatory diarrhea in Thailand and has been shown to cause diarrheal disease in ferrets, but it was much less invasive for epithelial cells in vitro than strain 81-176.[19] Portions of the genome[19] of *C. jejuni* strain CG8486 have been shown to be very similar to the genomes of *C. jejuni* strains 81-176[20] and NCTC 11168,[21] and here the CG8486 CPS locus will be described. The definition of the fine chemical structure of *C. jejuni* CG8486 CPS will allow for the design of glycoconjugate vaccine preparation(s) composed of this CPS and carrier protein(s) for immunological evaluation.

## 2. Experimental

### 2.1. Bacterial growth conditions and isolation of capsule polysaccharide

*C. jejuni* CG8486 was grown in Mueller–Hinton broth at 37 °C under a microaerophilic environment. Extraction of the CPS was achieved by stirring the cells in a hot water–phenol mixture for 2 h at 70 °C, followed by dialysis of the water layer, and then subjecting it to high-speed centrifugation to separate the insoluble LOS from the soluble CPS. The supernatant containing the CPS material was further purified through a size-exclusion column (Bio-Gel P-4), which yielded a single carbohydrate fraction as detected by the phenol–sulfuric acid assay.[22]

### 2.2. Sugar composition analysis and linkage analysis

Monosaccharide composition analysis was performed by the alditol acetate method.[23] Here, the glycosyl hydrolyses were carried out with 4 M-trifluoroacetic acid at 105 °C for 5 h followed by reduction in $H_2O$ with $NaBD_4$ overnight at room temperature, and subsequent acetylation with acetic anhydride at 100 °C for 2 h. The alditol acetate derivatives were analyzed by gas chromatography (GC) using a Varian 3400 gas chromatograph equipped with a 30 m DB-17 capillary column [210 °C (30 min)→240 °C at 2 °C/min], and by GC–mass spectrometry (GC–MS) in the electron-impact (EI) and chemical-ionization (CI) modes in a ThermoFinigan PolarisQ instrument. Sugar linkage analysis was achieved by the analysis of the permethylated alditol acetates, which were obtained by methylation,[24] hydrolysis,[23] reduction,[23] and acetylation.[23] The characterization of the permethylated alditol acetate derivatives was achieved by GC–MS in the electron impact mode (DB-17 column, isothermally at 190 °C for 100 min). The absolute configurations of the individual components were determined by the formation and characterization by GC–MS of the respective 2-(*S*)- and 2-(*R*)-butyl chiral glycosides.[25] The carbohydrate preparations were hydrolyzed in 4 M TFA at 100 °C for 4 h, reacted with 2-(*S*)- and 2-(*R*)-butanol at 100 °C for 6 h, and acetylated with acetic anhydride at 100 °C for 1 h. The 2-(*S*)- and 2-(*R*)-butyl chiral glycosides were characterized, and compared with standards, by GC–MS using a 30 m DB-23 capillary column isothermally at 200 °C.

### 2.3. Mass spectrometry

The molecular weight of *C. jejuni* CG8486 CPS was determined by matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF/MS), which was carried out on a MALDI Micro MX mass spectrometer. This instrument was operated in the linear mode with a $N_2$ laser source (337 nm) and positive ion detection. Samples for analysis were mixed with sinapinic acid matrix and 1–2 μL was deposited on a plate to dry (dry droplet method), and was then subjected to the spectrometer.

### 2.4. Nuclear magnetic resonance spectroscopy

$^1H$, $^{13}C$ and $^{31}P$ NMR spectra were recorded on a Bruker AMX 400 spectrometer at 293 K. Two-dimensional (2D) NMR correlation spectroscopy (COSY), total correlation spectroscopy (TOCSY), nuclear Overhauser enhancement spectroscopy (NOESY), heteronuclear spin quantum correlation (HSQC) spectroscopy experiments were performed using the instrument's Bruker software. Prior to performing the NMR experiments, the samples were lyophilized three times with $D_2O$

(99.9%). The HOD peak was used as the internal reference at $\delta_H$ 4.821 for the $^1H$ NMR spectroscopy, just before the NMR experiments were carried out, a $D_2O$ sample containing TMS ($\delta_H$ 0.00) was run to aid in the reference of the HOD signal. Orthophosphoric acid ($\delta_P$ 0.0) was used as the external reference for the $^{31}P$ NMR experiments.

### 2.5. Genetic analysis

The capsule biosynthesis locus of *C. jejuni* CG8486 was obtained during the whole genome sequencing of this strain using the Genome Sequencer 20, 454 sequencing instrument (Life Sciences, Branford, USA).[19] Assembly was performed by the NEWBLER ASSEMBLER software provided by the 454 Life Sciences and sequencer software (Gene Codes Corporation, Ann Arbor, MI). The open reading frames were identified using ARTEMIS software (http://www.sanger.ac.uk/Software/Artemis/). Identified CPS ORFs were locally aligned using BLASTN software (http://www.ncbi.nlm.nih.gov/BLAST/download.shtml) against the CPS genes of *C. jejuni* NCTC 11168 and 81-176. To correct potential pyrosequencing artifacts, primers were designed for conventional sequencing using dideoxy sequence analysis on an Applied Biosystems Model 3100 DNA sequencer. The capsule locus of CG8486 is part of whole genome shotgun project that has been deposited at DDBJ/EMBL/GenBank under the project accession AASY00000000.

## 3. Results

### 3.1. Monosaccharide composition and linkage analysis of *C. jejuni* CG8486 CPS

The monosaccharide composition analysis (Fig. 1) performed on the CPS of *C. jejuni* strain CG8486 revealed that it was composed of *N*-acetyl-glucosamine (GlcNAc) and a 6-deoxy-heptose (6d-Hep) ($MH^+ = 390$). In addition, a large peak belonging to an anhydro-heptose unit ($MH^+ = 243$) pointed to the presence of 1,7-anhydro-6-deoxy-heptose (1,7-anhydro-6d-Hep). The careful comparison between the alditol acetate derivative of a synthetically generated 6-d-L-*ido*-heptopyranose revealed that the 6d-Hep of *C. jejuni* CG8486 CPS possessed the idose configuration (6d-*ido*-Hep). The analysis of the 2-(*S*)- and 2-(*R*)-butyl chiral glycosides of 6d-*ido*-Hep and GlcNAc revealed that were present as D enantiomers.

Sugar linkage analysis showed that the 6d-D-*ido*-Hep was present as a 3-substituted unit [→3)-6d-Hep-(1→] and that the D-GlcNAc was present as a 4-substituted residue [→4)-GlcNAc-(1→]. Also detected, but in very low amounts, were 2,3-disubstituted 6d-*ido*-Hep [→2,3)-6d-Hep-(1→], and 3,7-disubstituted 6d-*ido*-Hep [→3,7)-6d-Hep-(1→] and terminal GlcNAc [GlcNAc-(1→]. Both units were present in the pyranose form.

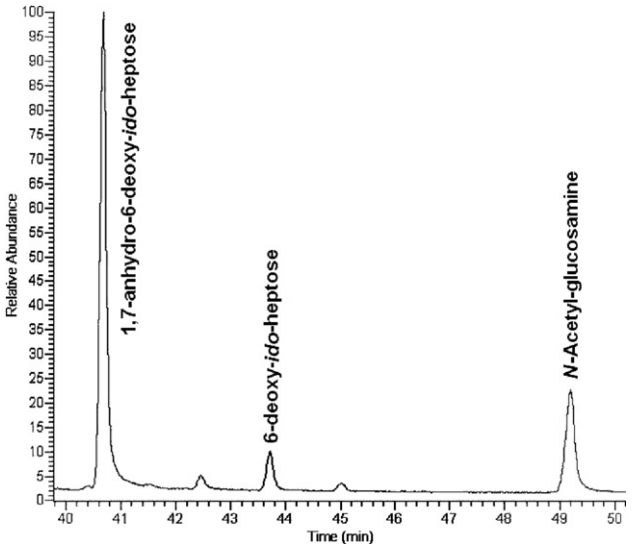



**Figure 1.** The GC–MS profile of the alditol acetate derivatives of *C. jejuni* CG8486 CPS.

### 3.2. NMR spectroscopy and mass spectrometry of *C. jejuni* CG8486 CPS

The $^1H$ NMR spectrum of the *C. jejuni* CG8486 CPS (Fig. 2) showed two anomeric signals at $\delta$ 4.83 (residue A) and $\delta$ 4.66 (residue B). The $^1H$ NMR spectrum also revealed one singlet at $\delta$ 2.06 assignable to the *N*-acetyl moiety of GlcNAc, and the methylene signals (multiplets) at $\delta$ 1.79 and $\delta$ 2.01 belonging to the 6-deoxy moiety of 6d-*ido*-Hep. A two-dimensional (2-D) $^1H$–$^1H$ COSY (Fig. 2), with the aid of selective 1D $^1H$–$^1H$ TOCSY experiments, allowed the assignment of the ring proton resonances of the two monosaccharide units. The anomeric signal A was assigned to the 6d-*ido*-Hep by virtue of its relationship with the H-6,6′ deoxy resonances (H-2 at $\delta$ 3.99; H-3 at $\delta$ 4.16; H-4 at $\delta$ 3.52; H-5 at $\delta$ 3.86; H-6,6′ at $\delta$ 1.80, 2.01; and H-7,7′ at $\sim\delta$ 3.72). Of particular note, the coupling constants (approximate values obtained from measuring the respective 2D COSY cross peaks) of the vicinal ring protons of the 6d-*ido*-Hep unit (A) ($J_{1,2} = 1.3$ Hz, $J_{2,3} = 1.0$ Hz, $J_{3,4} = 2.0$ Hz, $J_{4,5} = 1.8$ Hz) pointed toward an idose configuration, in agreement with the result previously obtained by monosaccharide analysis. The 6d-D-*ido*-Hep was determined to possess the β-configuration due to the observed *intra*-NOE connectivity between its H-1 and H-5 (Fig. 3). Accordingly, unit B with H-1 at $\delta$ 4.66 ($J_{1,2}$ 7.1 Hz) was assigned to β-D-GlcNAc (H-2 at $\delta$ 3.71; H-3 at $\delta$ 3.73; H-4 at $\delta$ 3.77; H-5 at $\delta$ 3.51; and H-6,6′ at $\delta$ 3.80, 3.91). A 2-D $^1H$–$^{13}C$ HSQC NMR experiment (Fig. 4) carried out on *C. jejuni* CG8486 CPS yielded two carbon anomeric resonances

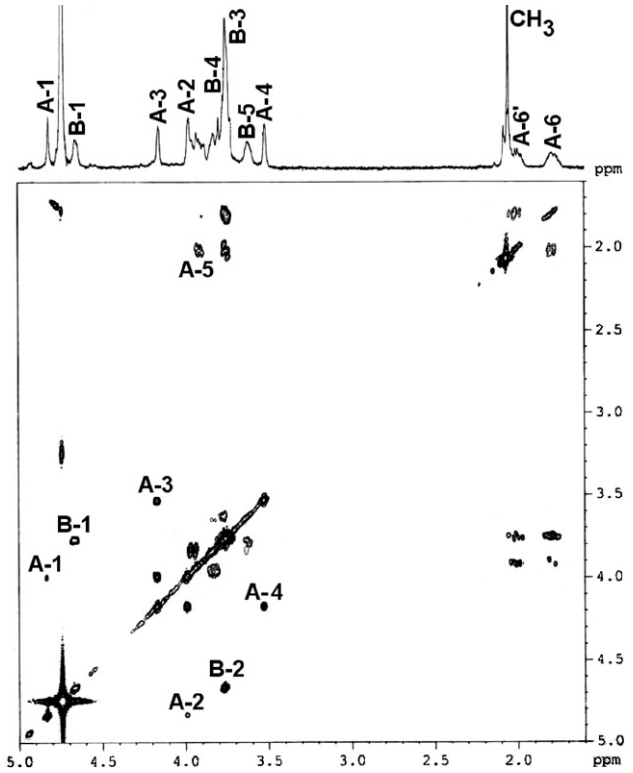



**Figure 2.** The 1D $^{1}$H (top) and 2D $^{1}$H–$^{1}$H COSY NMR spectra of *C. jejuni* CG8486 CPS.

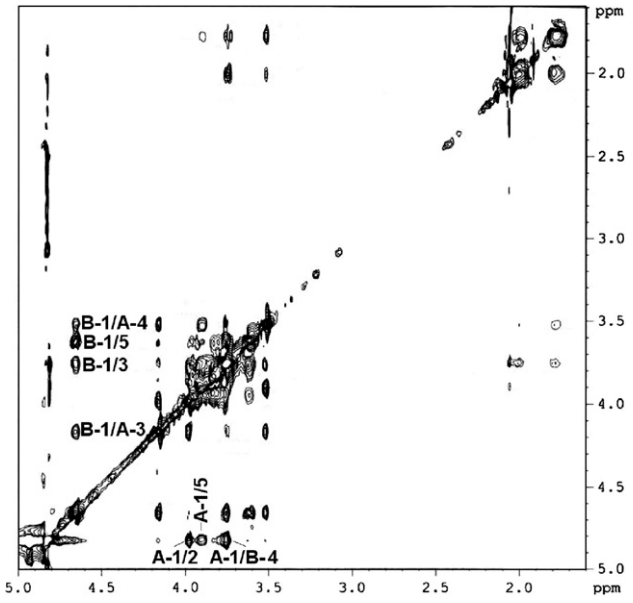



**Figure 3.** The 2D $^{1}$H–$^{1}$H NOESY NMR spectrum of *C. jejuni* CG8486 CPS.

at $\delta$ 99.2 for 6d-D-*ido*-Hep (A), and at $\delta$ 100.3 for GlcNAc (B). As anticipated, the carbons involved in the glycosidic linkages, C-3 ($\delta$ 77.0) of 6d-β-D-*ido*-Hep (A) and C-4 ($\delta$ 78.5) of β-D-GlcNAc (B), showed resonances in the low field range. The deoxy carbon resonance of 6d-β-D-*ido*-Hep could be readily observed at $\delta$ 32.60,

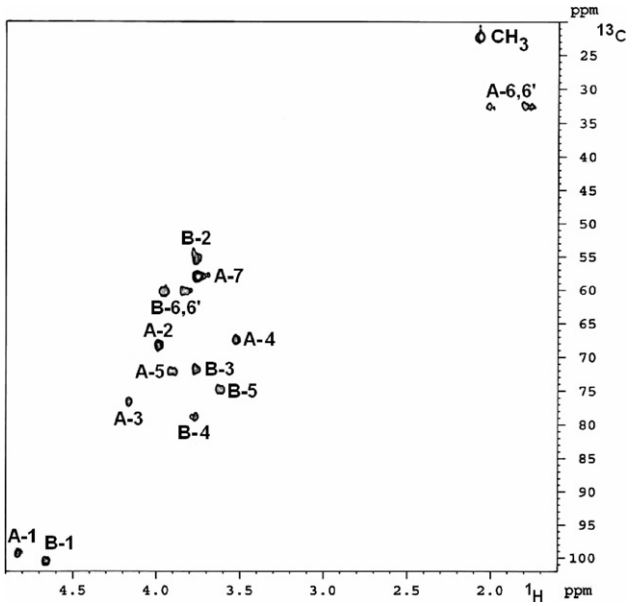


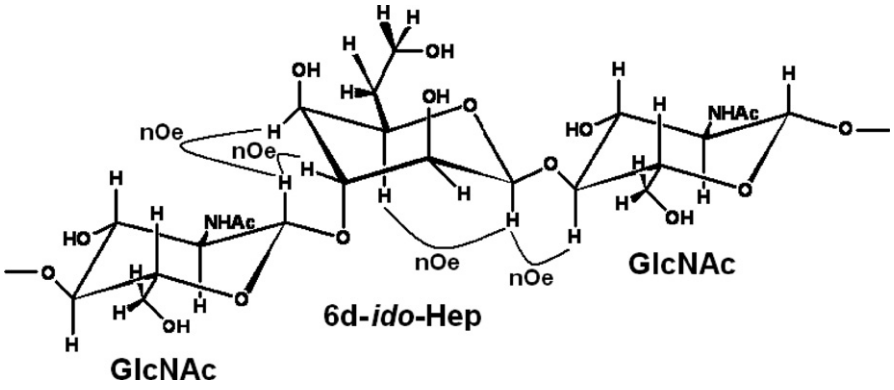

**Figure 4.** The 2D $^{1}$H–$^{13}$C HSQC NMR spectrum of *C. jejuni* CG8486 CPS.

and that of the acetyl methyl carbon of β-GlcNAc was detected at $\delta$ 22.1 (Fig. 4).

A 2-D $^{1}$H–$^{1}$H NOESY experiment (Fig. 3) revealed *inter*-NOE connectivities (illustrated below) between H-3 ($\delta$ 4.16) of residue A (3-substituted 6d-*ido*-Hep) and H-1 ($\delta$4.66) of residue B (4-substituted GlcNAc) for a →4)-β-D-GlcNAc-(1→3)-6d-β-D-*ido*-Hep-(1→ sequence. Also, the *inter*-NOE connectivity between H-4 ($\delta$ 3.52) of 4-substituted GlcNAc and H-1 ($\delta$ 4.83) of 3-substituted 6d-Hep confirmed the →3)-6d-β-D-*ido*-Hep-(1→4)-β-D-GlcNAc-(1→connection. Interestingly, an *inter*-NOE connectivity between H-1 of β-GlcNAc and H-4 ($\delta$ 3.52) of 6d-β-*ido*-Hep was also observed, which indicated that the adopted glycosidic bond conformation placed H-1 β-GlcNAc in close proximity to H-3 and H-4 of 6d-β-*ido*-Hep.

The $^{31}$P NMR spectrum (Fig. 5) of the CPS showed resonances at $\delta$ 14.27 (minor) and at $\delta$ 14.76 (major), typical of MeOPN groups, as previously detected in other *C. jejuni* CPSs.[7,9] As mentioned earlier, sugar linkage analysis revealed the presence of minor components of 2,3-disubstituted 6d-*ido*-Hep and 3,7-disubstituted 6d-*ido*-Hep, which, after examination of the $^{31}$P NMR spectrum, pointed to the fact that MeOPN groups may be attached to the O-2 position or O-7 position of a small number of 6d-*ido*-Hep units. Subsequently, linkage analysis performed on CPS that had been previously treated with 5% acetic acid at 100 °C, to cleave the MeOPN moieties, did not show any branched 6d-*ido*-Hep, but instead only 3-substituted 6d-*ido*-Hep was observed.

The MALDI-TOF-MS spectrum of *C. jejuni* CG8486 CPS showed a broad molecular ion at *m*/*z* 6500 Da,



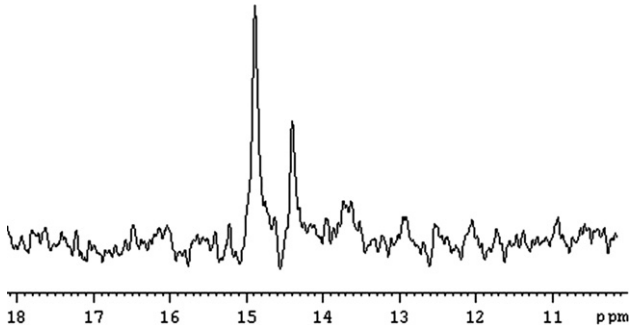




**Figure 5.** The $^{31}$P NMR spectrum of *C. jejuni* CG8486 CPS.

which pointed to approximately 17 disaccharide repeating units of [→3)-6d-β-D-*ido*-Hep-(1→4)-β-D-GlcNAc-(1→] (379.2 Da).

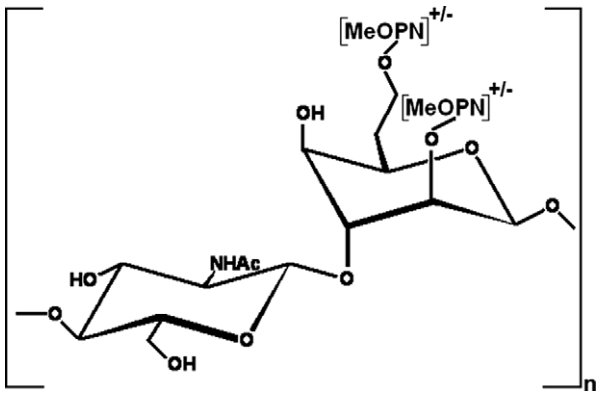
### 3.3. Analysis of the CPS genetic locus of *C. jejuni* CG8486

The variable region of *C. jejuni* CG8486 CPS locus is located between two clusters of genes encoding the capsule transport apparatus (Fig. 6 and Table 1). This region of the *C. jejuni* CG8486 chromosome between *kpsC* (Cj8484_1457c) and *kpsF* (Cj8486_1480c) is 26 kb in length and encodes 21 genes. There were CG8486 homologs of the genes that have been demonstrated to be involved in MeOPN synthesis and/or transfer in *C. jejuni* strain NCTC 11,168, namely, Cj1415c, Cj1416c, Cj1417c, and Cj1418c. *C. jejuni* CG8486 has two homologs of the MeOPN transferases reported in *C. jejuni* NCTC 11168, which is consistent with the attachment of MeOPN to two sites on the 6d-*ido*-Hep residues of the CG8486 capsule described here. These are Cj8486_1464c, which shows 46% identity to Cj1421c of *C. jejuni* NCTC 11168, which transfers MeOPN to C-3 position of the β-D-Gal*f*NAc residue of the NCTC 11168 capsule, and Cj8486_1475, which shows 48% identity to Cj1422c of *C. jejuni* NCTC 11168, which transfers MeOPN to the C-4 of D-*glycero*-α-L-*gluco*-heptopyranose of the NCTC 11168 capsule.[9] Interestingly, Cj8486_1475 is the only gene in the locus that is encoded on the opposite strand, and it is one of the three genes in the locus that appears to have the ability to undergo phase variation by slip strand mismatch repair (see Table 1).

The CG8486 CPS locus contained alleles of genes that have been shown to be involved in heptose biosynthesis in NCTC 11168 (*hddC*, *gmhA2*, *hddA* and *dmhA*). There are two predicted CG8486 proteins, Cj8486_1465c (92% identity) and Cj8486_1472c (27% identity), whose best match is HddC of NCTC 11168, a nucleotidyltransferase that activates D-*glycero*-D-*manno*heptose-1-P. Based on the degree of variation between these proteins, it is likely that Cj8486_1465c encodes the HddC involved in heptose biosynthesis. CG8486 has a divergent allele of a gene (Cj8486_1470c) encoding a putative protein annotated as a GDP-fucose synthetase in strains 81-176 and NCTC 11168. The predicted proteins encoded by Cj8486_1472c to Cj8486_1477c show limited homology to other *C. jejuni* genes.

## 4. Discussion

The covalent chemical structure of the CPS from *C. jejuni* strain CG8486 (serogroup HS:4) was determined to be composed of approximately 17 disaccharide repeating units (illustrated below) of 3-substituted 6d-β-D-*ido*-Hep and 4-substituted β-D-GlcNAc: [→3)-6d-D-β-



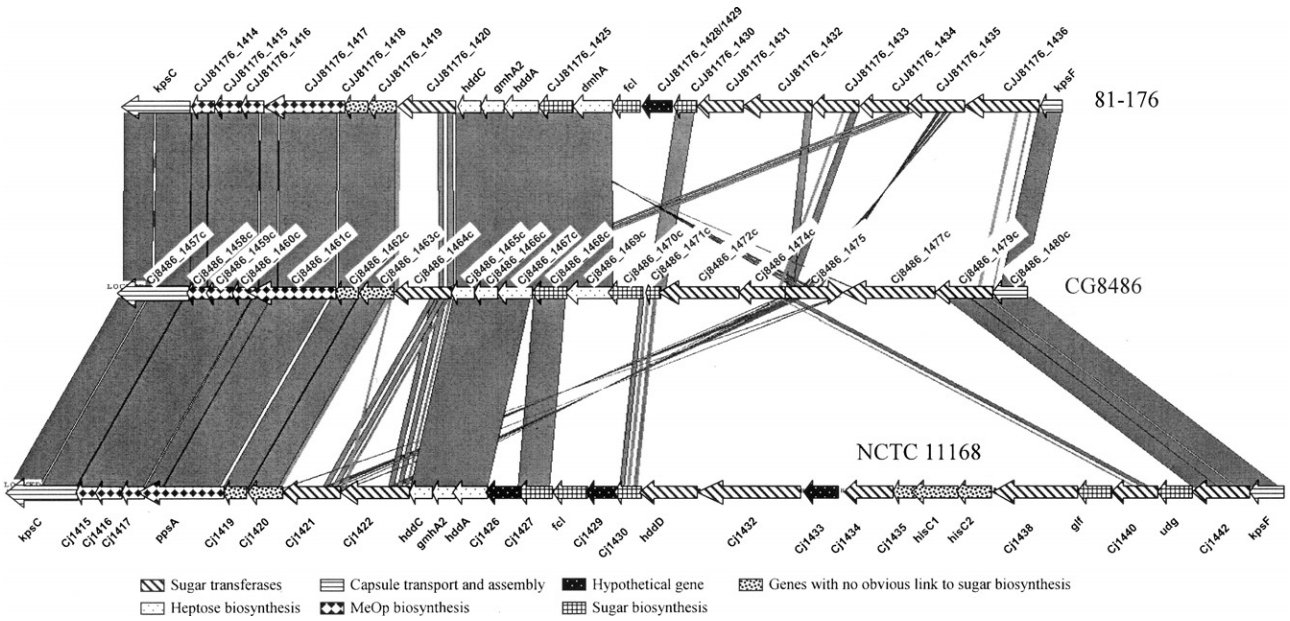



**Figure 6.** Comparison of the DNA of the capsule loci of *C. jejuni* strains 81-176, CG8486 and NCTC 11168. Comparison was made using the ARTEMIS Comparison tool software (http://www.sanger.ac.uk/Software/ACT/). The vertical block between CPS sequences represents conservation between sequences.

**Table 1.** Capsule biosynthesis genes of *C. jejuni strain CG8486*

| Locus tag | Putative function[a] | Closest relationship[b] | Identity[c] |
|---|---|---|---|
| Cj8486_1457c | Capsule polysaccharide export protein | *kpsC* (*C. jejuni* NCTC 11168) | 632/662 (95%) |
| Cj8486_1458c | Adenylylsulfate kinase | CJJ81176_1414 (*C. jejuni* 81-176) | 167/170 (98%) |
| Cj8486_1459c | Putative sugar-1-phosphate nucleotidyltransferase | Cj1416c (*C. jejuni* NCTC 11168) | 249/253 (98%) |
| Cj8486_1460c | Class I glutamine amidotransferase, putative | CJJ81176_1416 (*C. jejuni* 81-176) | 196/200 (98%) |
| Cj8486_1461c | Pyruvate kinase | Cj1418c (*C. jejuni* NCTC 11168) | 744/755 (98%) |
| Cj8486_1462c | Putative methyltransferase | Cj1419c (*C. jejuni* NCTC11168) | 252/253 (99%) |
| Cj8486_1463c* | Putative methyltransferase | CJJ81176_1419 (*C. jejuni* 81-176) | 255/257 (99%) |
| Cj8486_1464c | Putative sugar transferase | Cj1421c (*C. jejuni* NCTC 11168) | 250/542 (46%) |
| Cj8486_1465c | D-Glycero-D-manno-heptose 1-phosphate guanosyltransferase | *hddC* (*C. jejuni* NCTC 11168) | 203/220 (92%) |
| Cj8486_1466c | Sedoheptulose 7-phosphate isomerase | *gmhA2* (*C. jejuni* 81-176) | 197/201 (98%) |
| Cj8486_1467c | D-Glycero-D-manno-heptose 1-phosphate kinase | *hddA* (*C. jejuni* 81-176) | 332/339 (97%) |
| Cj8486_1468c | UDP-glucose 4-epimerase | CJJ81176_1425 (*C. jejuni* 81-176) | 310/312 (99%) |
| Cj8486_1469c* | GDP-Mannose 4,6-dehydratase | *dmhA* (*C. jejuni* 81-176) | 339/344 (98%) |
| Cj8486_1470c | GDP-Fucose synthetase | JJD26997_0705 (*C. jejuni* 269.97) | 206/344 (59%) |
| Cj8486_1471c | Putative nucleotide–sugar epimerase–dehydratase | CJJ26094_1482 (*C. jejuni* 260.94) | 173/181 (95%) |
| Cj8486_1472c | Putative sugar transferase | *hddC* (*C. jejuni* NCTC11168) | 166/603 (27%) |
| Cj8486_1474c | Galactosyltransferase | Cj1440c (*C. jejuni* NCTC 11168) | 197/386 (51%) |
| Cj8486_1475* | Putative sugar transferase | Cj1422c (*C. jejuni* NCTC 11168) | 297/617 (48%) |
| Cj8486_1477c | Putative sugar transferase | ggaB (*C. coli* RM2228) | 477/798 (59%) |
| Cj8486_1479c | Putative glycosyl transferase | Cj1442c (*C. jejuni* NCTC 11168) | 523/538 (97%) |
| Cj8486_1480c | Arabinose-5-phosphate isomerase | *kpsF* (*C. jejuni* 81-176) | 311/315 (98%) |

Locus tag entries followed by (*) indicate the presence of a G + C tract in the sequence of the gene suggestive of possible phase variation by slip strand mismatch repair. There is also a homopolymeric G + C tract in the intragenic region between Cj8486_1464 and Cj8486_1465.

[a] Functional attribution of CG8486 genes.

[b] Closest relationship based on the best BLASTP match on the non-redundant protein sequences database.

[c] The number of identical amino acids over the total number of amino acids in each protein is indicated. The number in parenthesis is the percentage of identity between the CG8486 protein and its best match in the protein database.

*ido*-Hep*p*-(1→4)-β-D-Glc*p*NAc-(1→], in which a small number of 6d-*ido*-Hep units were determined to carry a MeOPN group at the O-2 or O-7 position. The detection of small amounts of terminal GlcNAc suggests that this unit is present as the non-reducing end terminus of *C. jejuni* CG8486 CPS.

The ability of Campylobacter species to produce 6-deoxy-heptoses, many of unusual configurations, is a well-documented event.[2] 6-Deoxy-heptoses in the *altro*,[5] *talo*,[12] *gulo*,[13] and *galo*[14] configurations have been identified as members of Campylobacter CPSs. The identification of 6d-*ido*-Hep in this study revealed that *C. jejuni*

CG8486 (HS:4 CPS complex) also possessed a similar biosynthetic machinery for the production of unusual heptoses. Aspinall and co-workers[6] have also described a similar heptose, L-*glycero*-α-D-*ido*-heptopyranose, as a component of the CPS of *C. jejuni* strain ATCC 43431, which has been assigned to the HS:3 CPS complex. 6-Deoxy-heptose units, such as 6d-*man*-Hep,[26–29] 6d-*altro*-Hep,[30–32] and 6d-*talo*-Hep[33] have also been found in the PSs of other microorganisms.

The expression of MeOPN in *C. jejuni* CPSs has been recently described in detail.[9] This structural characteristic was also observed here, with the data showing that MeOPN may be present either at the O-2 position or at the exocyclic O-7 location. The CPS genetic locus of *C. jejuni* CG8486 was observed to contain two homologs (Cj8486_1464c and Cj8486_1475) of the MeOPN transferases reported in *C. jejuni* NCTC 11168[20] (CjNCTC11168_1421c and CjNCTC11168_1422c), which is consistent with the attachment of MeOPN to two sites on the 6d-*ido*-Hep residues of the CG8486 CPS described here. It should be noted that the presence of MeOPN was observed to vary between bacterial cell growths of CG8486. The *C. jejuni* CG8486 CPS locus is also similar to those of other *C. jejuni* strains that also contain deoxy-heptose modifications,[20,21] with Cj8486_1465c and Cj8486_1472c most likely being involved in the biosynthesis of 6d-*ido*-Hep. However, there are several genes that appear more divergent and may represent genes specific for the HS:4 CPS complex, such as the predicted proteins encoded by Cj8486_1472c to Cj8486_1477c.

A glycoconjugate vaccine composed of *C. jejuni* CG8684 CPS and the carrier protein $CRM_{197}$ has been synthesized and its protective efficacy against *C. jejuni* infection is presently being tested in animal models. The vaccinology results that will be obtained from this study will be published at a later date.

## Acknowledgments

This work was supported by a grant to M.A.M. from the National Sciences and Engineering Research Council of Canada. F.P. and P.G. were supported by the Military Infectious Disease Research Program, work unit 6000.RAD1.DA3.A0308.

## References

1. Galanis, E. *CMAJ* **2007**, *177*, 570–571.
2. Aspinall, G. O. *Carbohydr. Eur.* **1998**, *21*, 24–29.
3. Aspinall, G. O.; Fujimoto, S.; McDonald, A. G.; Pang, H.; Kurjanczyk, L. A.; Penner, J. L. *Infect. Immun.* **1994**, *62*, 2122–2125.
4. Aspinall, G. O.; McDonald, A. G.; Pang, H.; Kurjanczyk, L. A.; Penner, J. L. *Biochemistry* **1994**, *33*, 241–249.
5. Aspinall, G. O.; McDonald, A. G.; Pang, H. *Carbohydr. Res.* **1992**, *231*, 13–30.
6. Aspinall, G. O.; Lynch, C. M.; Pang, H.; Shaver, R. T.; Moran, A. P. *Eur. J. Biochem.* **1995**, *231*, 570–578.
7. McNally, D. J.; Jarrell, H. C.; Li, J.; Khieu, N. H.; Vinogradov, E.; Szymanski, C. M.; Brisson, J. R. *FEBS J.* **2005**, *272*, 4407–4422.
8. Kanipes, M. I.; Papp-Szabo, E.; Guerry, P.; Monteiro, M. A. *J. Bacteriol.* **2006**, *188*, 3273–3279.
9. McNally, D. J.; Lamoureux, M. P.; Karlyshev, A. V.; Fiori, L. M.; Li, J.; Thacker, G.; Coleman, R. A.; Khieu, N. H.; Wren, B. W.; Brisson, J. R.; Jarrell, H. C.; Szymanski, C. M. *J. Biol. Chem.* **2007**, *282*, 28566–28576.
10. Corcoran, A. T.; Annuk, H.; Moran, A. P. *FEMS Microbiol. Lett.* **2006**, *257*, 228–235.
11. Aspinall, G. O.; McDonald, A. G.; Pang, H.; Kurjanczyk, L. A.; Penner, J. L. *J. Biol. Chem.* **1993**, *268*, 18321–18329.
12. Aspinall, G. O.; Monteiro, M. A.; Pang, H. *Carbohydr. Res.* **1995**, *279*, 245–264.
13. Aspinall, G. O.; Monteiro, M. A.; Pang, H.; Kurjanczyk, L. A.; Penner, J. L. *Carbohydr. Res.* **1995**, *279*, 227–244.
14. Aspinall, G. O.; McDonald, A. G.; Pang, H.; Kurjanczyk, L. A.; Penner, J. *J. Biol. Chem.* **1993**, *268*, 6263–6268.
15. Yuki, N. *Muscle Nerve* **2007**, *35*, 691–711.
16. Bacon, D. J.; Szymanski, C. M.; Burr, D. H.; Silver, R. P.; Alm, R. A.; Guerry, P. *Mol. Microbiol.* **2001**, *40*, 769–777.
17. Guerry, P.; Szymanski, C. M.; Prendergast, M. M.; Hickey, T. E.; Ewing, C. P.; Pattarini, D. L.; Moran, A. P. *Infect. Immun.* **2002**, *70*, 787–793.
18. Guerry, P.; Shahida, B.; Monteiro, M. A. U.S. Patent Application 20,070,065,461, 2007.
19. Poly, F.; Read, T.; Tribble, D. R.; Baqar, S.; Lorenzo, M.; Guerry, P. *Infect. Immun.* **2007**, *75*, 3425–3433.
20. Karlyshev, A. V.; Champion, O. L.; Churcher, C.; Brisson, J. R.; Jarrell, H. C.; Gilbert, M.; Brochu, D.; St. Michael, F.; Li, J.; Wakarchuk, W. W.; Goodhead, I.; Sanders, M.; Stevens, K.; White, B.; Parkhill, J.; Wren, B. W.; Szymanski, C. M. *Mol. Microbiol* **2005**, *55*, 90–103.
21. Karlyshev, A. V.; Ketley, J. M.; Wren, B. W. *FEMS Microbiol. Rev.* **2005**, *29*, 377–390.
22. Dubois, M.; Gilles, K. A.; Hamilton, J. K.; Rebers, P. A.; Smith, F. *Anal. Chem.* **1956**, *28*, 350–356.
23. Sawardeker, J. S.; Sloneker, J. H.; Jeanes, A. *Anal. Chem.* **1965**, *37*, 1602–1604.
24. Ciucanu, I.; Kerek, F. *Carbohydr. Res.* **1984**, *131*, 209–217.
25. Leontein, K.; Lindberg, B.; Lonngren, J. *Carbohydr. Res.* **1978**, *62*, 359–362.
26. Hellerqvist, C. G.; Lindberg, B.; Samuelsson, K.; Brubaker, R. R. *Acta Chem. Scand.* **1972**, *26*, 1389–1393.
27. Komandrova, N. A.; Gorshkova, R. P.; Isakov, V. V.; Ovodov, Y. S. *Bioorg. Khim.* **1984**, *10*, 232–237.
28. Knirel, Y. A.; Paramonov, N. A.; Shashkov, A. S.; Kochetkov, N. K.; Yarullin, R. G.; Farber, S. M.; Efremenko, V. I. *Carbohydr. Res.* **1992**, *233*, 185–193.
29. Gilbert, M.; Mandrell, R. E.; Parker, C. T.; Li, J.; Vinogradov, E. *Chembiochem.* **2007**, *8*, 625–631.
30. Hoffman, J.; Bengt Lindberg, B.; Lönngren, J. *Carbohydr. Res* **1976**, *47*, 261–267.
31. Kondo, W.; Sato, N.; Ito, T. *Carbohydr. Res.* **1979**, *70*, 117–123.
32. Hoffman, J.; Lindberg, B.; Skaug, N.; Hofstad, T. *Carbohydr. Res.* **1980**, *84*, 181–183.
33. Perry, M. B.; MacLean, L. L.; Schollaardt, T.; Bryan, L. E.; Ho, M. *Infect. Immun.* **1995**, *63*, 3348–3352.