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Synthesis of the 6-*O*-Methyl-D-*glycero*-α-L-*gluco*-heptopyranose Moiety Present in the Capsular Polysaccharide from *Campylobacter jejuni* NCTC 11168

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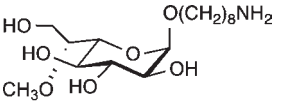
ABSTRACT



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The first synthesis of the 6-*O*-methyl-D-*glycero*-α-L-*gluco*-heptopyranose moiety present in the capsular polysaccharide from *Campylobacter jejuni* NCTC 11168 is reported. The target (1) was synthesized as the 8-aminooctyl glycoside and then conjugated to bovine serum albumin (BSA) for the generation of antibodies recognizing this motif. Heptose 1 was obtained from D-galactose via a series of galactofuranose derivatives.

Campylobacter jejuni is an important food-borne pathogen that can cause severe enteritis and is a leading cause of diarrheal disease.[1] Infections by *C. jejuni* are also linked to Guillain–Barré syndrome, a neurological disorder that results in paralysis of varying severity, and which is occasionally fatal.[2] The outer surface of all campylobacters is functionalized with a species-specific capsular polysaccharide (CPS), the production of which is required for virulence.[3] The biochemical pathways that assemble the CPS are therefore potential sites for therapeutic intervention.

As part of a larger program directed toward understanding polysaccharide biosynthesis in different campylobacter species,[4] we have focused our attention on *C. jejuni* NCTC11168 (HS:2), which possesses a CPS with a tetrasaccharide repeating unit (Figure 1).[5] This structure contains a number of unusual motifs including an *N*-acetylgalactosamine residue in the furanose ring form, a glucuronic acid residue amidated with 2-aminoglycerol, a methyl phosphoramidate moiety, and a 6-*O*-methyl-heptose residue possessing the D-*glycero*-L-*gluco* stereochemistry.

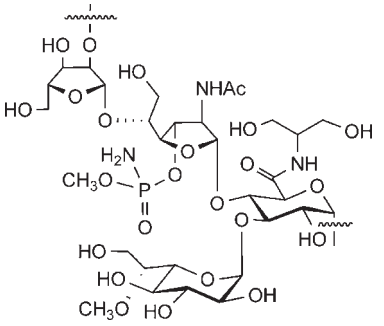

Figure 1. Structure of the tetrasaccharide repeating unit from *C. jejuni* NCTC 111168.

Heptose sugars are present in a number of bacterial species, particularly in the core structures of lipopolysaccharides from Gram-negative bacteria.[6] However, the

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CPS from *C. jejuni* NCTC11168 is the first naturally occurring glycoconjugate reported to contain a heptose with the D-*glycero*-L-*gluco* stereochemistry.[7] It is of interest to synthesize glycoconjugates incorporating this heptose for the generation of antibodies specific to this novel motif, as well as to probe the various biosynthetic enzymes (e.g., glycosyltransferases) involved in assembling the full-length glycan. We describe here the first synthesis of this monosaccharide, functionalized with an aminooctyl spacer arm (**1**, Figure 2), and its attachment to a protein carrier (BSA) for the generation of antibodies.

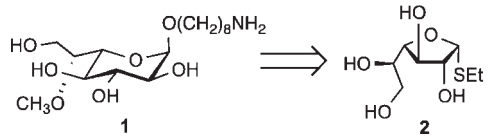

Figure 2. Retrosynthetic analysis of **1**.

Because heptoses are not widely available from natural sources, their chemical synthesis has been the topic of a number of investigations.[6,8,9] The majority of heptose syntheses involve chain extension of an oxidized hexose derivative, possessing an aldehyde group at C-6, via Grignard or Wittig reactions. Subsequent functionalization of this homologated product provides the target molecule. In the case of D-*glycero*-L-*gluco*-heptose, using this approach would require access to significant quantities of L-glucose, which is not readily available. Therefore, we investigated a less widely used strategy, involving chain extension from the C-1 aldehyde functionality of a hexose, in particular D-galactose. Although the synthesis of heptoses by this route has been reported as far back as investigations by Fischer and Kiliani on the synthesis of cyanohydrins,[10] the stereoselectivity of these processes is generally not high and it is has not been widely used in modern organic chemistry.[6] Mindful of this, we envisioned that the target, **1**, could be synthesized from galactofuranosyl thioglycoside **2** (Figure 2), which can be obtained on large scale from D-galactose in two steps,[11] provided that chain extension could be performed in a stereoselective manner.

The synthesis of **1** is illustrated in Scheme 1. Thioglycoside **2**, obtained from D-galactose via a two-step route involving formation of the corresponding diethyl dithioacetal and subsequent cyclization,[11] was first treated with dimethoxypropane and *p*-toluenesulfonic acid. This reaction afforded the expected product **3** in 88% yield. Benzylation of the two hydroxyl groups using benzyl bromide and sodium hydride (giving **4**) followed by hydrolysis of the acetal provided **5** in 93% yield over the two steps. The resulting diol was then protected at the primary position as a *tert*-butyldiphenylsilyl ether affording, in 95% yield, **6**.

With a route to secondary alcohol **6** in place, treatment with methyl iodide and sodium hydride in DMF resulted in introduction of the methyl group, and then the silyl ether was replaced with a benzyl ether via a process involving desilylation and alkylation. The product **9** was obtained in 92% yield over the three steps from **6**. Attempts to prepare **9** in two steps from diol **5** by formation of a stannylene acetal, selective benzylation of O-6,[12] and methylation led to a lower overall yield of the product than the approach outlined in Scheme 1. Hydrolysis of the thioglycoside was achieved by reaction with NBS and water in acetone, affording hemiacetal **10** in 95% yield.

The key step in the route was the chain extension of **10**, which was successfully achieved upon reaction with divinyl zinc, generated in situ from vinyl magnesium bromide with $ZnBr_2$.[13] Divinylzinc addition to **10** led to the formation of **11** in 91% yield with excellent stereoselectivity due to chelation control. None of the other isomer could be detected. To explore the importance of the $ZnBr_2$ to the stereocontrol of the reaction, we also treated **10** with vinyl magnesium bromide alone or combined with $MgBr_2 \cdot Et_2O$.[14] Both reactions provided the product with poor selectivity and in reduced yield. The structure of **11** was proven by ^1H NMR spectroscopy of a subsequently synthesized cyclic intermediate and the preparation of a crystalline derivative (see below).

Ozonolysis of the alkene in **11** afforded the heptose derivative **12**, which was O-acetylated and then treated with *p*-toluenethiol and $BF_3 \cdot OEt_2$, yielding thioglycoside **14** in 81% yield over three steps from **11**. The β-isomer of **14** could be separated by chromatography, and the stereochemistry was confirmed by ^1H NMR spectroscopy in $CDCl_3$; the $^3J_{1,2}$ was 10.1 Hz, indicative of a *trans* relationship between H-1 and H-2.

Because the final target, **1**, possesses an axially oriented aglycone, it was necessary to convert the acetate ester on O-2 into a nonparticipating benzyl group. This was achieved in two steps by deacetylation (yielding **15**) and benzylation resulting in a 91% yield of **16**. This thioglycoside is expected to be a suitable donor for the preparation of glycoconjugates containing this unusual monosaccharide. We note that **16** was obtained from **2** in a comparable number of steps to

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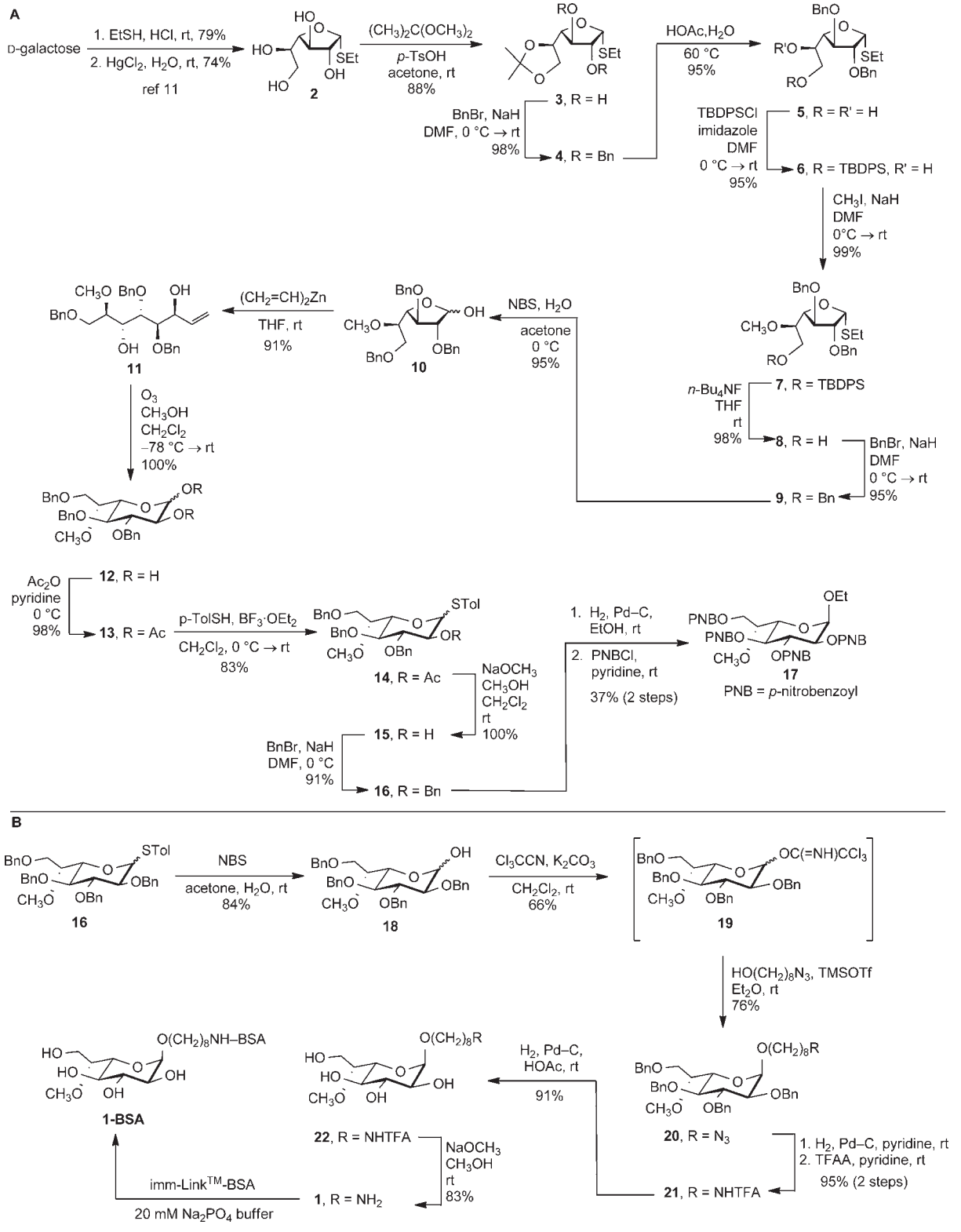
Scheme 1. Synthesis of **1** and its bovine serum albumin conjugate (**1-BSA**)



that recently reported for the *de novo* synthesis of heptose glycosyl donors.[9]

Further support for the stereochemistry of the heptose ring substituents was obtained by the preparation of a crystalline derivative of **16**. This was achieved by first treating **16*β*** with hydrogen over a palladium on carbon catalyst in ethanol. Unexpectedly, this reaction afforded a product in which the four benzyl ethers had been cleaved and the thioglycoside moiety had been replaced by an ethoxy group, undoubtedly from the ethanol used as the

reaction solvent. Subsequent treatment of this product with *p*-nitrobenzoyl chloride in pyridine gave the product **17** in 37% yield over the two steps. The product was recrystallized from ethyl acetate–hexane, and X-ray analysis of the resulting crystals confirmed the D-*glycero*-L-*gluco* stereochemistry (CCDC deposition number 838206; see Supporting Information).

Having unequivocally established the stereochemistry of **16**, its reaction with 8-azidooctanol in the presence of NIS and silver triflate[15] was attempted. Unexpectedly, this reaction led to a mixture of products in which the undesired β-glycoside (1,2-*trans*-glycoside) predominated. This problem could be circumvented (Scheme 1B) by conversion of **16** into the corresponding reducing sugar **18** and, in turn, the trichloroacetimidate **19**. Glycosylation of **19** with 8-azidooctanol promoted by TMSOTf[16] led to the formation of **20** (95% yield), as a 4:1 α/β mixture of glycosides, which could be separated by chromatography.

The conversion of **20** into **1** in a single step by treatment with hydrogen over a palladium on carbon catalyst proved problematic. Very low yields of impure product were obtained in a range of solvents including ethyl acetate, ethanol, acetic acid, and 1:1 ethanol–acetic acid. Therefore, we explored a stepwise approach, which we had used when a similar problem arose in the course of another investigation.[17] Thus, the azido group in **20** was reduced by hydrogenation over a palladium catalyst poisoned by pyridine. The resulting amine was converted to the corresponding *N*-trifluoroacetamide derivative **21** in 95% overall yield. Hydrogenolysis of the benzyl ethers in **21** proceeded without difficulty, and the resulting product **22** was obtained in 91% yield. Finally, cleavage of the amide in **22** was carried out by reaction with sodium methoxide giving an 83% yield of **1**. Subsequent reaction of **1** with amine-reactive BSA led to the expected glycoconjugate with an incorporation of four heptose residues per protein.

In summary, this report describes the first synthesis of the D-*glycero*-L-*gluco*-heptose monosaccharide present in the CPS of *C. jejuni* NCTC11168. The route involved the chain extension of a readily obtained galactofuranose derivative (**10**), which was then converted into the target **1**. This approach therefore differs from the usual approach used for the synthesis of heptoses, where chain extension proceeds via a C-6 oxidized hexose derivative. Further investigations on the use of donors such as **16** and **19** in the preparation of more complex glycoconjugates containing this heptose moiety are under investigation.

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Supporting Information Available. Experimental details and ^{1}H and ^{13}C NMR spectra for new compounds. X-ray crystallographic information for **17**. This material is available free of charge via the Internet at http://pubs.acs.org

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