One-pot synthesis of a pentasaccharide with antibiotic activity against *Helicobacter pylori*†

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A pentasaccharide that contains the α-1,4-GlcNAc mucin core two-branched *O*-glycan has been synthesized by a one-pot, two-step glycosylation strategy; this particular carbohydrate motif may provide protection against *H. pylori* induced pathologies since the synthetic pentasaccharide inhibits cholesterol α-glucosyltransferase (IC_{50} of 0.47 mM).

Helicobacter pylori infects about half of the world's population. Three percent of infected patients develop peptic ulcers, gastric cancer and mucosa-associate lymphoma.[1] It has been proposed that mucosal *O*-glycans that contain a terminal α-1,4-linked *N*-acetylglucosamine can inhibit *H. pylori* growth by inhibition of α-glucosyl cholesterol transfer.[2] Inhibition of α-glucosyltransferase by α-1,4-GlcNAc capped synthetic oligosaccharides may constitute a novel therapeutic approach to tackle *H. pylori* infections selectively.[2] This enzyme is essential for the survival of the bacterium and inhibitors would act as antibiotics. To determine the structural features responsible for *H. pylori* growth inhibition, the activity of defined oligosaccharides has to be assessed. Therefore, we synthesized pentasaccharide **1** (Fig. 1). Here we report a one-pot,[3] two-step regioselective glycosylation sequence as key to the synthesis of pentasaccharide **1**. The synthetic oligosaccharide exhibited good inhibitory activity against cholesterol α-glucosyltransferase.

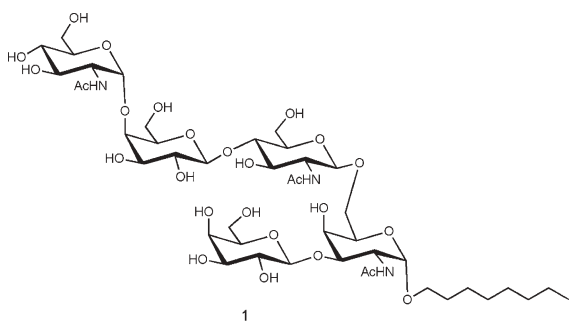

Fig. 1 Structure of pentasaccharide **1**.

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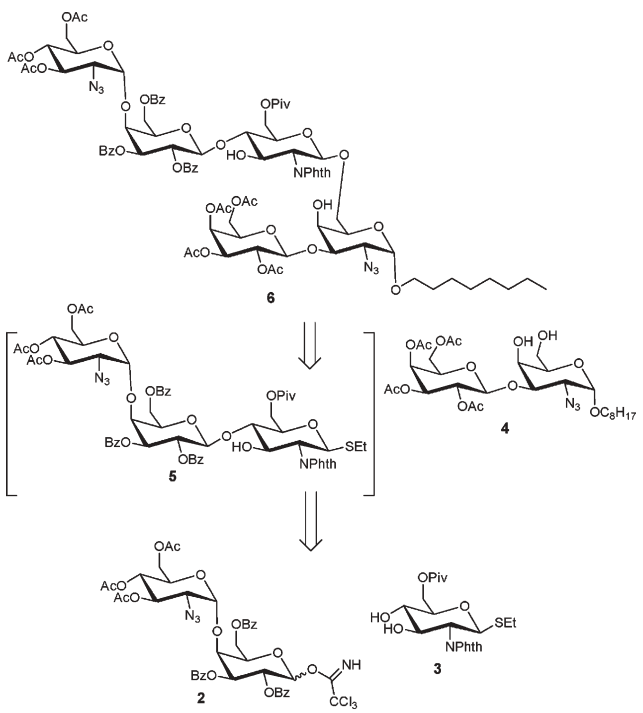
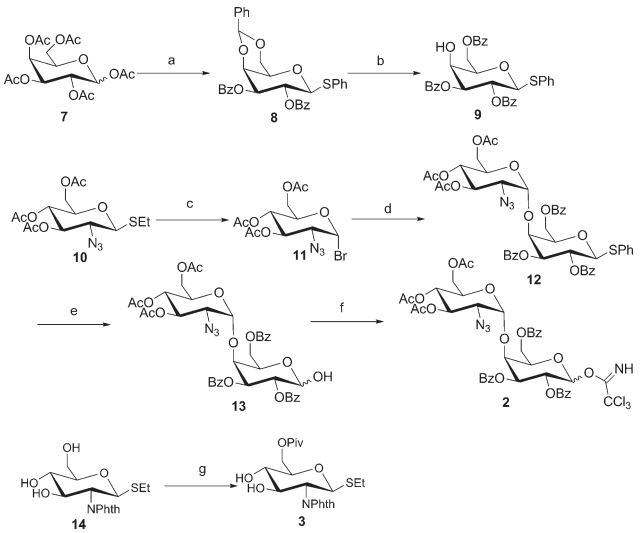
Retrosynthetic analysis of target pentasaccharide **1** revealed fully protected pentasaccharide **6** as a precursor to the final structure (Scheme 1). Formation of masked pentasaccharide **6** was to be achieved *via* a one-pot sequence envisioned to involve initially the union of disaccharide trichloroacetimidate **2** and glucosamine monosaccharide **3** to form trisaccharide thioglycoside **5**, followed by *in situ* addition of disaccharide **4**.

Based on this retrosynthetic analysis, the assembly of oligosaccharide **1** commenced with the synthesis of disaccharide **12** (Scheme 2). Following literature precedence,[4] glycosyl bromide **11** served in the formation of the α-1,4-GlcNAc linkage. Phenylthio galactoside **9** was prepared starting from galactose pentaacetate **7**[5] by removal of the benzoyl group and regioselective benzylation of **8**.

Glucosamine bromide building block **11**[4] was synthesized by *in situ* activation of thioglycoside **10**[4] with bromine.[6] Silver triflate promoted union of glycosylating agent **11** and acceptor **9** gave disaccharide **12**. Conversion of **12** to the corresponding disaccharide trichloroacetimidate **2** proceeded in 70% yield over two steps. Building block **3** was equipped with a C6 pivaloyl group but exhibits two potential acceptor hydroxyl groups on C3 and C4.



Scheme 1 Retrosynthetic analysis of pentasaccharide **1**.

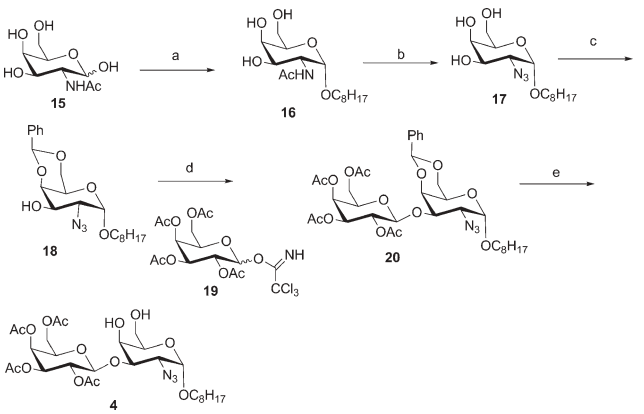


Scheme 2 Preparation of disaccharide **2** and monosaccharide **3**. *Reagents and conditions*: (a) ref. 11; (b) (i) 70% HOAc, 40 °C; (ii) Py, BzCl, CH_2Cl_2, −30 °C, 2 h, 75%; (c) Br_2, CH_2Cl_2, 0 °C, 30 min; (d) **9**, AgOTf, *sym*-collidine, CH_2Cl_2, −30 °C, 4 h, 76%; (e) NBS, THF/H_2O, r.t., 90%; (f) CCl_3CN, CH_2Cl_2, K_2CO_3, 0 °C to r.t., 78%; (g) Py, PivCl, CH_2Cl_2, 82%.

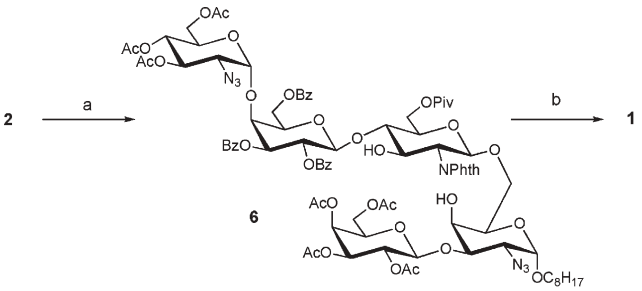
The C2 *N*-phthalimido group drastically lowers the reactivity of the C3 hydroxyl to yield mainly the 1–4 linked product.[4,7]

Galactosamine **15** was converted into the corresponding α-octyl glycoside by heating with $BF_3{\cdot}OEt_2$[8] to 70 °C for 3 h in octanol as solvent (Scheme 3). The amine was converted into the corresponding azide **17**.[9] Placement of a 4,6-benzylidene group by treatment with benzaldehyde dimethyl acetal and a catalytic amount of $TsOH{\cdot}H_2O$ gave monosaccharide **18**. Union of glycosyl building block **19** and nucleophile **18** furnished initially the orthoester before addition of more TMSOTf[10] transformed the orthoester to the desired disaccharide **20**. Hydrolysis of the benzylidene group yielded disaccharide diol **4**.

With the three building blocks, **2**–**4**, in hand, the key reaction sequence *en route* to the target pentasaccharide was executed in



Scheme 3 Synthesis of disaccharide **4**. *Reagents and conditions*: (a) $BF_3{\cdot}OEt_2$, CH_2Cl_2, octanol, 70 °C, 2 h, 78%; (b) (i) 1 M NaOH, 120 °C, 12 h; (ii) TfN_3, MeOH, K_2CO_3, $CuSO_4$, CH_2Cl_2/H_2O, r.t., overnight, two steps 75%; (c) *p*-$TsOH{\cdot}H_2O$, dimethoxytoluene, CH_3CN, 80%; (d) **19**, TMSOTf, CH_2Cl_2, 0 °C to r.t., 2 h, 83%; (e) 70% HOAc, 60 °C, 3 h, 78%.



Scheme 4 One-pot procedure for the pentasaccharide. *Reagents and conditions*: (a) (i) **3**, TMSOTf, CH_2Cl_2, −70 °C, 1 h; (ii) **4**, NIS/TfOH, CH_2Cl_2, −50 to −10 °C, 2 h, 63%; (b) (i) PPh_3, THF/H_2O, r.t.; (ii) $NH_2CH_2CH_2NH_2$, CH_3CN–EtOH–toluene, 80 °C, 18 h; (iii) Py, Ac_2O, r.t.; (iv) 1 M NaOMe, MeOH, 2 days, four steps, 70%.

one pot (Scheme 4).[3] Disaccharide **2** was activated with TMSOTf to react exclusively with the C4 hydroxyl group of monosaccharide **3**. Addition of disaccharide acceptor **4** to the reaction mixture, followed by NIS/triflic acid to activate the thioglycosyl group of the *in situ* formed trisaccharide completed the sequence. The fully protected pentasaccharide **6** was obtained in 63% yield from two disaccharides and one monosaccharide. Four deprotection steps were required to liberate the target molecule of all masking groups:[12] reduction of the azide, removal of the *N*-phthaloyl group, acetylation in pyridine and acetic anhydride and removal of acetate esters and benzoate esters by exposure to sodium methoxide in methanol provided pentasaccharide target **1** in 70% yield over four steps.

Inhibition assays comparing different synthetic oligosaccharides for their ability to inhibit the activity of cholesterol α-glucosyltransferase revealed that **1** was significantly more active than other closely related carbohydrates. The IC_{50} was determined at 0.47 mM.[2b]

In summary, we have synthesized a pentasaccharide implicated as a potent antibiotic against *H. pylori*. Key to this synthesis was the one-pot glycosylation sequence to assemble the main carbohydrate scaffold. The final product proved to be a good inhibitor of cholesterol α-glucosytransferase. Further tests of the antibiotic activity of the synthetic oligosaccharide in mice are currently under way and will be reported in due course.

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