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 (IC₅₀ 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 7⁵ 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/H₂O, r.t., 90%; (f) CCl₃CN, 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 BF₃·OEt₂⁸ 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·H₂O gave monosaccharide **18**. Union of glycosyl building block **19** and nucleophile **18** furnished initially the orthoester before addition of more TMSOTf¹⁰ transformed the orthoester to the desired disaccharide **20**. Hydrolysis of the benzylidene group yielded disaccharide **14**.

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) TfN₃, MeOH, K₂CO₃, CuSO₄, CH₂Cl₂/H₂O, r.t., overnight, two steps 75%; (c) *p*-TsOH·H₂O, dimethoxytoluene, CH₃CN, 80%; (d) **19**, TMSOTf, CH₂Cl₂, 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) PPh₃, THF/H₂O, r.t.; (ii) NH₂CH₂CH₂NH₂, CH₃CN–EtOH–toluene, 80 °C, 18 h; (iii) Py, Ac₂O, 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 IC₅₀ 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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