Chemical and Chemo-Enzymatic Approach to the Synthesis of P^k-trisaccharide Deoxy Analogues

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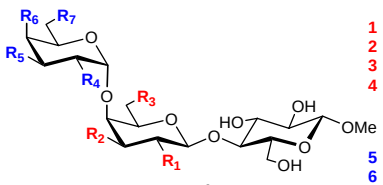
INTRODUCTION

Shiga toxin and shiga-like toxins (Stxs or Verotoxins) can initiate clinical complications in humans infected by bacteria that express the toxins.[1] Shiga-like toxins from *E. coli*, can be divided into two subgroups, Stx1 and Stx2. They consist of an enzymatically active A subunit and a cell-adhesion carrier, B5.[1] The crystal structure for Stx1 B-pentamer complexed with a P^k-trisaccharide analogue showed three different carbohydrate binding sites.[2] Two peripheral binding sites, 1 and 2, are more important than site 3, which is closer to the centre of the pentamer.

Among other methods deoxy analogues of P^k-trisaccharide were used for evaluation of the relative affinity of the binding sites.[3] On the other hand, the only crystal structure available for the more biologically significant Stx2 shows no bound carbohydrate.[4] Furthermore, it is known that the affinity of Stx2 for the P^k-trisaccharide is significantly less than that of Stx1, and attempts to design inhibitors of Stx2 have encountered significant difficulties.

We propose to use deoxy analogues of P^k-trisaccharide for mapping the putative binding sites of Stx2 by FTICR-MS. We present here the synthesis of native P^k-trisaccharide **1** and deoxy analogues **2**-**4**.



1

1 $R_1=R_2=R_3=OH$
2 $R_1=H$, $R_2=R_3=OH$
3 $R_1=R_3=OH$, $R_2=H$
4 $R_1=R_2=OH$, $R_3=H$

5 $R_4=H$, $R_5=R_6=R_7=OH$
6 $R_4=OH$,$R_5=H$, $R_6=R_7=OH$
7 $R_4=R_5=OH$, $R_6=H$, $R_7=OH$
8 $R_4=R_5=R_6=OH$, $R_7=H$

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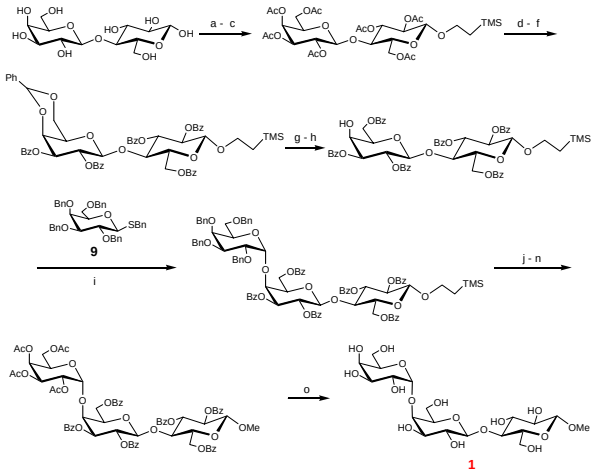
2. Paton, J., Paton, A. *Clinical Microbiology Reviews 11*(3), **1998** , 450
3. Nyholm, P., Magnusson, G., Zheng, Z., Norel, R., Binnington-Boyd, B., Lingwood, C.A. *Chem. & Biol. 3*(4), **1996**, 263
4. Fraser, M. E., Fujinaga, M., Cherney, M. M., Twiddy, E. M., James, M. N. G. *J. Biol. Chem. 279*, **2004** , 27511

ACKNOWLEGEMENTS

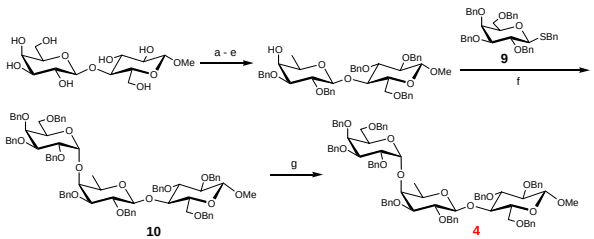
Financial support was provided by the Natural Sciences and Engineering Research Council of Canada (NSERC) and the Alberta Ingenuity Centre for Carbohydrate Science (AICCS).

Scheme 1. Synthesis of Native P^k-trisaccharide



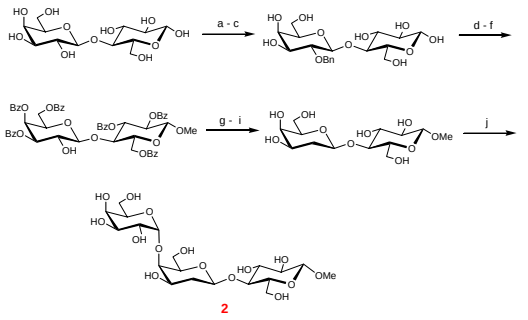
Reagents and Conditions : a) NaOAc, Ac_2O b) 45% HBr, DCM, 97% c) $HOCH_2CH_2TMS$, $HgBr_2$,$Hg(CN)_2$,CH_3CN,43% d) NaOMe, MeOH, 98% e) $PhCH(OMe)_2$, *p*-TsOH, CH_3CN f) BzCl, Py, 33% (2steps) g) H_2, $Pd(OH)_2$, MeOH/DCM, 72% h) BzCl, Py, 0°C, 73% i) **10**, NIS, TfOH, 3Å MS, DCM, 70% j) H_2, $Pd(OH)_2$, MeOH/DCM, 100% k) Ac_2O, Py, 74% l) TFA, DCM, 92% m) Cl_3CCN, DBU, DCM, 93% n) MeOH, BF_3OEt_2, 47% o) NaOMe, 65%.

Scheme 2. Synthesis of 6'-Deoxy Analogue



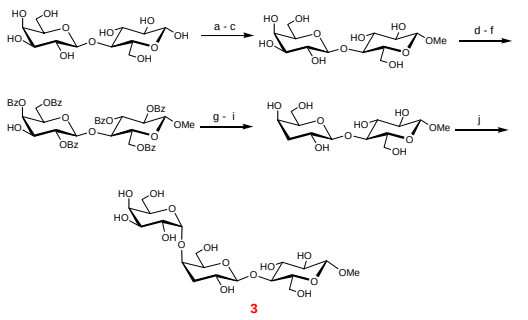
Reagents and Conditions : a) $PhCH(OMe)_2$, CSA, DMF, b) BnBr, NaH, DMF, 69% (2 steps) c) 80% AcOH, 97% d) MsCl, Py, 0°C, 74% e) $LiAlH_4$, THF, reflux, 97 % f) **10**, 3Å MS, DCM, 92% g) H_2, $Pd(OH)_2$, MeOH, 91%.

Scheme 3. Synthesis of 2'-Deoxy Analogue



Reagents and Conditions : a) $Me_2CH(OMe)_2$, TsOH, 80°C, 30% b) BnBr, NaH, DMF, 89% c) AcOH, reflux, 76% d) BzCl, DMAP, Py, reflux, 32% e) 45% HBr, DCM, 72% e) MeOH, 66% g) 1,1'-Thiocarbonyldiimidazole, Toluene, reflux, 74% h) AIBN, Tributhyltin hydride, 60% i) NaOMe, MeOH, 94% j) 4-OH-Glu-epimerase/α(1-4)-Gal-transferase Fusion Enzyme, UDP-Glc, DTT, HEPES pH 8.2 Buffer, Alkaline Phosphatase.

Scheme 4. Synthesis of 3'-Deoxy Analogue



Reagents and Conditions : a) NaOAc, Ac_2O b) MeOH, BF_3OEt_2, 65% c) MeOH, NaOMe, 97% d) i) Dibuthyltin oxide, 3Å MS, CH_3CN, reflux ii) Tetrabuthylammonium bromide, AllBr, 78% e) BzCl, Py, 90% f) AcOH, $PdCl_2$, MeOH, 69% g) 1,1'-Thiocarbonyldiimidazole, toluene, reflux,41% h) AIBN, Tributhyltin hydride, toluene, 58% i) NaOMe, MeOH, 92% j) 4-OH-Glu-epimerase/α(1-4)-Gal-transferase Fusion Enzyme, UDP-Glc, DTT, HEPES pH 8.2 Buffer, Alkaline Phosphatase.

RESULTS AND FUTURE WORKS

Synthesis of the target compounds involves 2 key steps: α-galactosylation of a lactose derivative and deoxygenation of selected hydroxyl groups. The first step may be accomplished by chemical or enzymatic procedures. The choice of deoxygenation method is mainly dictated by the position of the hydroxyl group and nature of protecting group pattern.

For the synthesis of native P^k-trisaccharide **1** and 6'-deoxy analogue **4** chemical glycosylation were chosen (Scheme 1 and 2). Catalytic hydrogenation of **10** gives the target 6'-deoxy analogue **4**. The 2' and 3'-deoxy analogues are synthesized by chemo-enzymatic reaction and Barton-McCombie deoxygenation (Scheme 3 and 4). In the future, we will synthesize the other deoxy analogues **5**-**8** and the binding of deoxy analogues to Stx2 will be evaluated by Surface Plasmon Resonance (SPR) and Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (FTICR-MS).