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Unique tetrameric and hexameric mannoside clusters prepared by click chemistry

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ABSTRACT

The synthesis of novel tetrameric and hexameric mannoside clusters bearing 1,2,3-trizole linkages via Cu(I)-catalyzed azide–alkyne cycloaddition reaction ("click chemistry") is described. An attractive feature of these multiarmed mannoside clusters as potential inhibitors of uropathogenic *Escherichia coli* is the use of an aglycone whose length is designed to fit in the tyrosine gate. The acetylated mannosides were deprotected and the corresponding de-*O*-acetylated mannosides were found to exhibit good water solubility.

1. Introduction

It has been widely accepted that the interactions between carbohydrates and their protein receptors play a crucial biological role in cell recognition and adhesion.[1] Unfortunately, the interaction of single monosaccharide or oligosaccharides with their protein receptors is weak with dissociation constants in the millimolar to micromolar range.[2] However, the presence of multiple copies of the carbohydrate (multivalency) strengthen the binding[3,4] such that synthetic ligands can compete with the natural multivalent ligands.

Urinary tract infections (UTIs) are one of the most common bacterial infections, affecting millions of people around the world each year.[5–7] They are mainly caused by strains of gram negative uropathogenic bacteria, *Escherichia coli* (UPEC).[8] The vast majority of *E. coli* express Type 1 fimbriae or pili, which are hair-like protein structures present on the cell surfaces. UPEC bind to the urinary tract endothelial surface through the 200 to 500 fimbriae which extend from the surface of each bacterial cell. At the tip of each pili there is an adhesin protein known as FimH, a lectin that recognizes terminal α-D-mannopyranoside residues of the *N*-linked glycoprotein Uroplakin Ia (UPIa) on the endothelial surface, enabling adherence and invasion of the host cell and at the same time preventing the rapid clearance of *E. coli* from the UTI by the bulk flow of urine. UPIa is a membrane glycoprotein that is expressed abundantly on superficial umbrella cells of the urinary tract on the endothelial surface.[9] It was found that the mannose-binding pocket of FimH is adjacent to a hydrophobic area known as the tyrosine gate that is formed by two tyrosines (Tyr48 and Tyr137) and one isoleucine (Ile52).[10] In binding the UPI oligosaccharides that are terminated by trimannosides, the tyrosine gate accommodates the non-polar faces of the non-terminal mannopyranoses.[11,12] The combination of a mannose-binding pocket with an adjacent extended hydrophobic region on FimH has resulted in compounds that are designed to bind to this site being poorly recognized by the many other mannose-binding receptors in humans.[13] Binding occurs by a catch-bond mechanism that is critical for attachment in the high-sheer environment of the urinary tract.[14,15] Infection commences with FimH binding to UPIa that triggers a conformational change in the cell surfaces,[16] allowing the bacteria to enter and establish sub-surface colonies that are difficult to eradicate.

Blocking the FimH-mediated bacterial adhesion is sufficient to prevent bacterial entry and infection and a variety of α-D-mannopyranosides have been synthesized to evaluate the utility of anti-adhesion strategies against *E. coli*. Firon et al. found that phenyl α-D-mannopyranosides, particularly the 2-chloro-4-nitro derivative, bound effectively,[17] and recently Hultgren and coworkers have shown that α-linked biphenyl derivatives are even more effective.[18] Other phenyl glycosides with planar substituents on the phenyl ring have also proved to be quite effective,[19–21] and these compounds remain attached to FimH surface long enough to be clinically relevant.[22] In 2005, Bouckaert et al. discovered[10] that heptyl α-D-mannopyranoside bound to FimH with strengths comparable to the better phenyl glycosides.

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Multivalent glycoconjugates have attracted considerable attention as anti-pathogenic agents because of their improved binding characteristics.[23] In particular, those derived from mannose have been synthesized and investigated as inhibitors of bacterial adhesion by different research groups.[24–37] We aim to expand the repertoire of available polyvalent mannosides in order to determine which scaffolds are most compatible with the FimH environment. We have chosen to use linker arms matching the heptyl glycoside that binds well to FimH[10,12] as others have done with different scaffolds.[35] It was thought that the open approximately spherical shape of the mannose-tipped dendrimers constructed here would provide opportunities to binding to FimH from more bacteria than would similarly-linked mannosides that were attached to one rim of a cyclodextrin.[35]

The Cu(I)-catalyzed click reaction between terminal alkynes and azides to form a stable 1,4-disubstituted trizole product with high regioselectivity in excellent yield under mild reaction conditions has proven to be a powerful tool in the preparation of glycodendrimers.[38] A number of mannose-tipped dendrimers have been prepared using this reaction at some stage of their synthesis.[24,25,28,30,31,35,37] Herein, we report the syntheses of unique tetrameric and hexameric mannoside clusters built on hydroquinone and 1,1,1-tris(4-hydroxyphenyl)ethane scaffolds by a convergent strategy using click chemistry.

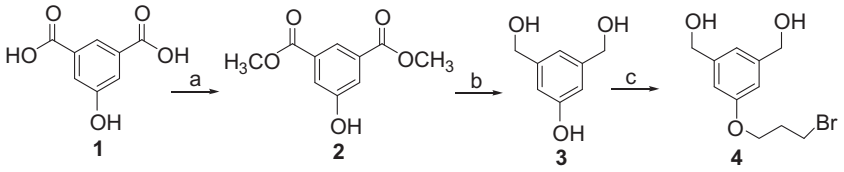
2. Results and discussion

2.1. Synthesis of alkynyl-terminated dendritic cores *9* and *10*

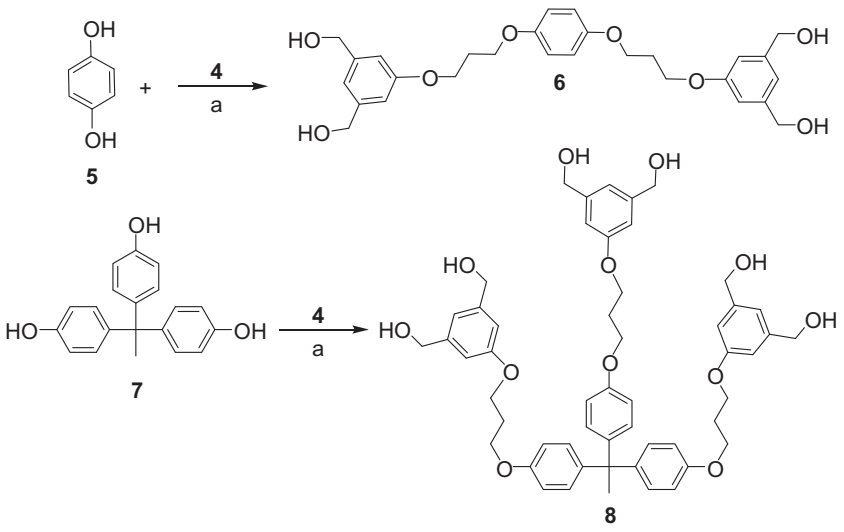
The synthesis of useful bromide **4** is depicted in Scheme 1. 5-Hydroxyisophthalic acid **1** was converted to the corresponding dimethyl 5-hydroxyisophthalate **2** using a catalytic amount of H_2SO_4 in refluxing methanol in 85% yield. Reduction of the diester **2** by $LiAlH_4$ in refluxing THF provided the known benzyl alcohol **3** in 92% yield.[39] Compound **3** was then selectively *O*-alkylated at the phenolic position using 1,3-dibromopropane, K_2CO_3, and a catalytic amount of KI in refluxing acetone to give the corresponding bromo derivative **4** in good yield. Its ^{1}H-NMR spectrum contained two triplets at 4.09 and 3.64 ppm for the CH_2OAr and CH_2Br groups, respectively. The structure was also confirmed by the presence of peaks at 66.0 ppm for the CH_2OAr group and 33.2 ppm for the CH_2Br group in the ^{13}C-NMR spectrum. It should be noted that 1,3-dibromopropane was used in excess to avoid the formation of undesired dimeric or oligomeric products.

The tetrol **6** and hexol **8** were obtained by the reaction of phenols **5** and **7** with bromide **4** in the presence of K_2CO_3 and a catalytic amount of KI in refluxing acetone in yields of 69% and 70%, respectively (Scheme 2). The ^{1}H-NMR spectra confirmed the structure of alkylated phenols **5** and **7** from **4** by the disappearance of the triplet at 3.64 ppm of the CH_2Br group. The ^{13}C-NMR spectra were a good indicator for the formation of polyols **6** and **8**, as they showed the appearance of a new peak around 64.0 ppm for the CH_2OAr group and the disappearance of the peak at 33.2 ppm that belongs to the CH_2Br group.

Finally, the propargyl ethers **9** and **10** were obtained by the reaction of compounds **6** and **8** with propargyl bromide and NaH in dry DMF at low temperature in yields of 62% and 60%, respectively as outlined in Scheme 3. A new signal, a narrow triplet, appeared in the ^{1}H-NMR spectra of each of the product propargyl ethers **9** and **10** at about 2.5 ppm corresponding to the acetylenic proton and a new narrow doublet appeared at about 4.2 ppm corresponding to the propargylic CH_2. The ^{13}C-NMR spectra also provided evidence for the formation of ethers **9** and **10**, as they contained peaks for acetylenic carbons at 79.7 ppm and about 74 ppm.



Scheme 1. Synthesis of bromide **4**. Reagents and conditions: (a) CH_3OH, H_2SO_4/reflux, 85%; (b) $LiAlH_4$,THF, reflux, 92%; (c) K_2CO_3, acetone, $Br(CH_2)_3Br$, reflux, 24 h, 78%.



Scheme 2. Synthesis of benzyl alcohols **6** and **8**. Reagents and conditions: (a) **4**, acetone, K_2CO_3, KI(cat), reflux, 24 h, 69% for **6** and 70% for **8**.