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Synthesis of CifiDABCO and mannose co-functionalized G(4)-PAMAM dendrimer for use in antibiotics

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Synthesis of $C_{16}$DABCO and mannose co-functionalized G(4)-PAMAM dendrimer for use in antibiotics

Harrison Wesley VanKoten
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Synthesis of $\text{C}_{16}\text{DABCO}$ and mannose co-functionalized $\text{G}(4)$-PAMAM dendrimer for use in antibiotics

I. Abstract

Synthesis of $\text{C}_{16}\text{DABCO}$ and mannose co-functionalized $\text{G}(4)$-PAMAM dendrimer is expected to yield a useful antimicrobial that is effective in binding to mannose specific lectins on bacterial fimbriae. Thus far, mannose has been attached to the dendrimer with near complete functionalization. $\text{C}_{16}\text{DABCO}$ and mannose co-functionalized $\text{G}(4)$-PAMAM dendrimer have been synthesized, but not in sufficient quantities to allow examination of its the antibacterial properties. Future work includes optimizing the final synthesis and performing toxicity studies.

II. Introduction

Antibiotics are useful tools in the medical field because they can mean the difference between life and death of an individual. Inappropriate use of antibiotic, increases an evolved bacterial resistance to the antibiotic decreasing its effectiveness. As resistance of pathogens to current antimicrobial agents increases, new antimicrobials are urgently needed\textsuperscript{1}. Consequently, design and synthesis of antibiotics that circumvent to bacterial resistance are becoming increasingly necessary.

The first step in bacterial infection is the binding of bacterial lectins to carbohydrate moieties on the membrane of the host. This is accomplished via lectins on proteinaceous extensions of the bacteria (fimbriae) binding to carbohydrates in
the glycocalyx of the host cell\textsuperscript{2}. Glycodendrimers (artificial carbohydrates) inhibit this interaction by competitively binding to bacteria\textsuperscript{3}.

The present study focuses on inhibiting host cell binding by introducing glycodendrimers to bacteria. Synthesizing a compound that binds bacterial lectins and prevents bacteria from interacting with to host cells is not antibiotic. For it to be considered an antibiotic, it must bind to the bacterium and cause a cascade of events that ultimately lead to bacterial death. To accomplish the proposed cascade leading to bacterial death, the mannose functionalized PAMAM dendrimer will be "armed" with quaternary ammonium groups (DABCO)\textsuperscript{4}. Derivitizing the mannose-functionalized dendrimer with quaternary ammonium salts may result in an effective antibiotic with a low chance of bacteria gaining resistance.

Mannose-specific lectins found on the fimbriae of \textit{Escherichia coli}, for example, bind to mannans of the cellular glycocalyx\textsuperscript{5}. Small mannose-containing glycodendrimers are 10 to 100-fold more potent as inhibitors of the interaction between \textit{E. coli} and mannans than methyl mannosid\textsuperscript{5}. Galabiose (galα1-4gal) binds to \textit{Streptococcus suis}, which can cause meningitis in humans\textsuperscript{6}. Also, galabiose-functionalized glycodendrimers were shown to inhibit \textit{S. suis D282} mediated hemagglutination of human erythrocytes with a 50 to 170 fold increase in potency relative to monomeric galabiose control compounds. Gastric infection by \textit{Helicobacter pylori} is mediated by blood group antigens and as such serves as another example of a lectin/carbohydrate adhesion process for which glycodendrimer application could be beneficial\textsuperscript{7}. 
Figure 1. Schematic representation of inhibition by glycodendrimers of bacterial adhesion to the glycocalyx of the host cell. The spider web shaped structure surrounding the bacterium is the Mannose-DABCO co-functionalized G4 PAMAM dendrimer. This diagram is meant to represent the Mannose residues preventing bacterial adhesion to the host cell.
III. Results

The following is a scheme of the synthesis performed and a table of yields from the synthesis. The synthesis were performed in duplicate unless otherwise noted. The discussion section will cover both trial, while the experimental section will cover the method with the highest yield.

**Scheme 1. Synthesis of C₁₆DABCO and mannose co-functionalized dendrimer**

1. $\text{Ac}_2\text{O}, \text{In(OTf)}_3$
2. $\text{NH}_2\text{NH}_2/\text{AcOH}$
3. $\text{CCl}_3\text{CN}$

(22%, 3 steps)

1. $\text{G(4) PAMAM}$
2. DMSO, rt
3. NaOMe/MeOH

(80%, 2 steps)

1. TsCl, pyr
2. $\text{N}^+\text{C}_{16}\text{H}_{33}$
3) Hydrolysis of remaining Tosyl Groups.
Table 1. Yields obtained from synthesis performed.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Yield 1</th>
<th>Yield 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,2,3,4,6-penta-O-acetyl-(\alpha)-D-mannopyranoside (1)</td>
<td>74%</td>
<td>59%</td>
</tr>
<tr>
<td>2,3,4,6-tetra-O-acetyl-(\alpha)-D-mannopyranoside (2)</td>
<td>20%</td>
<td>42%</td>
</tr>
<tr>
<td>2,3,4,6-tetra-O-acetyl-(\alpha)-D-mannosyl trichloroacetimidate (3)</td>
<td>85%*</td>
<td>89%*</td>
</tr>
<tr>
<td>2-(2-isothiocyanatoethoxy)ethanol (4)</td>
<td>&lt;1%</td>
<td>54%</td>
</tr>
<tr>
<td>1-O-(5-isothiocyanato-3-oxapentyl)-2,3,4,6-tetra-O-acetyl-(\alpha)-D-mannopyranoside (5)</td>
<td>N/A</td>
<td>24%</td>
</tr>
<tr>
<td>Mannose Functionalized Dendrimer (G4) (6)</td>
<td>61%</td>
<td>N/A**</td>
</tr>
<tr>
<td>C_{16}DABCO and Mannose Co-functionalized Dendrimer (G4) (7)</td>
<td>N/A</td>
<td>N/A</td>
</tr>
</tbody>
</table>

* Product was not purified. **Only one reaction was performed.
IV. Discussion

The objectives of this research were to synthesize C$_{16}$DABCO and mannose co-functionalized G(4)-PAMAM dendrimer and perform toxicity studies on bacteria and eukaryotic cells. The synthesis of C$_{16}$DABCO and mannose co-functionalized G(4)-PAMAM dendrimer is still being optimized. Therefore, toxicity studies have not yet been performed.

The difference between the two yields for compound 1 could be due to the ice bath procedure. In the first trial, the reaction was allowed to sit in the ice bath and very slowly warm up to room temperature. In the second trial, the reaction was removed from the ice bath before the In(OTf)$_3$ or Indium triflate was added. If heat was a product of the reaction, this would likely lead to the observed decrease yield in the second trial. Also, in the second trial, when the bicarbonate was added, the reaction swelled uncontrollably, and ran on to the workbench. The spill was a small portion of the reaction. Most of the it stayed in the round bottom flask. This undoubtedly lead to a decrease in yield. In the synthesis of compound 2, the reaction was warmed to 85°C in the first trial rather than to 55 °C as should have occurred due to unfamiliarity with equipment. This could have lead to effecting the yield in trial one.

Instead of several washings, 150 mL of water, saturated aqueous NaHCO$_3$ solution, and brine were combined in a single 450 mL aqueous washing, producing layers that could not be separated. An additional set of washings was needed (75 mL of EtOAc, 2 x 100 mL of saturated NaHCO$_3$ solution, 100 mL of brine). In the second trial, the aqueous layer was extracted with EtOAc (50 ml). The organic layers were
combined then washed with water (3 x 75 ml), sat. aqueous NaHCO₃ solution (3 x 75 ml), and brine (3 x 75 ml). This resulted in a better yield.

In the synthesis of compound 3, the procedure worked well. However, an increased yield of product might have been obtained by rinsing the silica plug with an additional 50 mL of EtOAc to ensure all products were removed from the plug. The yields reported are for the crude products of both reactions. Both trials were combined because there was not enough product from either trial to continue to the next synthetic step. Both trials were purified by column chromatography. Following chromatography, most of the fractions with the desired product were not pure but instead were mixtures. The pure fractions were combined. An additional column might have yielded more of the desired product. Perhaps another ratio of eluent would also yield better results.

The synthesis of compound 4 was performed twice because the yield from the first procedure was so low. Column chromatography was not needed on compound 4, but was performed in trial 1 in an attempt to increase purity. Instead, a silica plug would suffice. In addition, trial 1 was subjected to a Kugelrohr distillation, which caused significant product decomposition. In the second trial, the procedure was followed as described in the experimental section. The Kugelrohr distillation was deemed unnecessary in purification of the product. Rather than purifying the product the Kugelrohr led to a decreased yield.

The synthesis of compound 5 was readily accomplished, however, purification of this compound was more involved. TLC plates showed streaking, even after altering the ratio of solvents that were used in the eluent. Very few of the
fractions from column chromatography contained the purified product. The final mass of the product from the first trial was not recorded, and therefore its yields are not listed in the results section because there appeared to be no product. NMR spectroscopy suggests, the reaction was contaminated with water or other reaction-suppressing contaminants, causing production of side products that were difficult to separate from the desired product.

The synthesis of compound 6 was readily accomplished. After the initial exposure to NaOMe or Sodium methoxide, most of the acyl groups were still on the mannosides as shown in the NMR spectrum (data not included). After an additional reaction with NaOMe, the NMR showed no trace of any acyl groups. Millipore water was not used in the dialysis. Instead, DI water was used because Millipore water was not available at the time. This could have led to a decrease in yield. The duration of dialysis was longer than described in the procedure below. The extra time most likely allowed the mannose-functionalized dendrimer to escape through the pores in the dialysis tubing. A longer dialysis was performed in an attempt to increase the purity of the desired product. The yield for trial 1 was good enough that a second trial was not needed.

Several attempts were made in the present study to synthesize compound 7. Tosylation of the mannose-functionalized dendrimer is not the problem, but rather the attachment of C_{16}DABCO to the mannose units. In the first attempt, the reaction was done in pyridine. The H\textsuperscript{1} NMR showed several pyridine peaks suggesting that pyridine was replacing the tosyl groups instead of the C_{16}DABCO. When the reaction was attempted a second time in DMF, solubility became an issue. Eventually the
solvent was changed to DMSO. After the reaction was completed, the $H^1$ NMR did not show any $C_{16}$DABCO peaks. However, resonances consistent with pyridinium functionalization of the dendrimer were also not present. Therefore, that problem had been solved.

All of the synthetic steps up to the final one has been accomplished in respectable yields. Further research will include optimizing the final synthetic step and determining if compound 7 had any antibacterial properties.
V. Experimentals

Synthesis of Mannose-Functionalized G4 PAMAM dendrimer with terminal C$_{16}$-DABCO
1,2,3,4,6-penta-O-acetyl-\(\alpha\)-D-mannopyranoside (1). A solution of \(\alpha\)-D-mannose (7.99 g, 44.4 mmol) and acetic anhydride (135 mL, 1.43 mmol) was cooled to 0°C. While the reaction was stirring on a stir plate, indium triflate (1.2579 g, 2.24 mmol) was added. The reaction was allowed to warm to room temperature. After 1 h, EtOAc (200 mL) and 10% aq. Na\(_2\)CO\(_3\) solution (200 mL) were added and stirred for another h. The organic layer was isolated, washed with saturated aqueous NaHCO\(_3\) solution (2 x 75 ml), dried over MgSO\(_4\), and filtered over activated charcoal (in EtOAc). The EtOAc was evaporated \textit{in vacuo} to afford 12.85 g (74% yield) yellow oil. The \(^1\)H NMR spectrum agreed with previously reported values\(^8\).

2,3,4,6-tetra-O-acetyl-\(\alpha\)-D-mannopyranoside (2). A solution of \textbf{1} (10.2313 g, 26.211 mmol), hydrazine acetate (2.897 g, 31.45 mmol), and DMF (30 ml) was stirred at 55°C for 50 minutes. EtOAc (50 ml) and water (50 ml) were added. Sat. aqueous NaHCO\(_3\) (100 ml) was required for separation of the two layers. The aqueous layer was extracted with EtOAc (50 ml). The organic layers were combined then washed with water (3 x 75 ml), sat. aqueous NaHCO\(_3\) solution (3 x 75 ml), and
brine (3 x 75 ml). The organics were then dried over MgSO₄ and the EtOAc was removed *in vacuo* to give 3.842 g (42%) of highly viscous, slightly yellow oil. ¹H NMR (500 MHz, CDCl₃) δ 5.37 (ap dd, 1H, 3.3 Hz, H-1), 5.18-5.28 (m, 3H), 4.18-4.23 (m, 2H), 4.09-4.12 (m, 1H), 3.80 (bs, 1H, OH), 2.12 (s, 3H, COCH₃), 2.07 (s, 3H, COCH₃), 2.01 (s, 3H, COCH₃), 1.96 (s, 3H, COCH₃) ppm. The ¹H NMR spectrum agreed with previously reported values⁸.

2,3,4,6-tetra-O-acetyl-α-D-mannosyl trichloroacetimidate (3). A solution of (2) (3.8241 g, 10.98 mmol), trichloroacetonitrile (3.75 ml, 37.33 mmol), and CH₂Cl₂ (40 ml) was cooled to 0°C. DBU (.0745 ml, 0.045 mmol) was added drop-wise to the solution. After 3 hours of stirring, the solvent was removed *in vacuo* and a dark brown residue was filtered through a silica plug (3:2 Hexanes:EtOAc). Two batches were combined to give a total mass of 7.54g (85% yield). The solution was concentrated and purified by silica gel chromatography (3:2 Hexanes:EtOAc) Fractions were combined and concentrated to give 4.8253 g of pure product. ¹H NMR (500 MHz, CDCl₃) δ 8.75 (s, 1H, NH), 6.14 (d, 1H, H-1), 5.32 (m, 1H), 5.22-5.30
(m, 2H), 3.96-4.17 (m, 3H), 2.06 (s, 3H), 1.95 (s, 3H), 1.93 (s, 3H), 1.87 (s, 3H) ppm. The $^1$H NMR spectrum agreed with previously reported values.$^8$

\[
\text{SCN} \quad \text{O} \quad \text{OH}
\]

2-(2-Isothiocyanatoethoxy)ethanol (4). A solution of thiophosgene (5.4 ml, 70 mmol) and CH$_2$Cl$_2$ (200 ml) was cooled to 0°C. A solution of 2-(2-aminoethoxy)ethanol (7 ml, 70 mmol), triethylamine (20 ml, 140 mmol), and CH$_2$Cl$_2$ (400 ml) was added via syringe over the course of 30 minutes (syringe pump) while stirring. Upon the completion of the addition of the amino alcohol, the solvent was removed in vacuo. The resulting residue was dissolved in water (100 ml) and extracted with CH$_2$Cl$_2$ (2 x 100 ml). The organic layers were combined, washed with water (2 x 50 ml), dried over MgSO$_4$, and then concentrated in vacuo. The resulting residue was filtered through a silica plug (1:1 Hexanes:EtOAc) to give 5.5481 g (54%) of viscous yellow oil. $^1$H NMR (500 MHz, CDCl$_3$) δ 3.72 (t, 2H, HOCH$_2$), 3.62-3.68 (m, 4H, CH$_2$OCH$_2$), 3.58 (t, 2H, SCNCH$_2$), 2.34 (s, 1H, OH) ppm. The $^1$H NMR spectrum agreed with previously reported values.$^8$. 
1-O-(5-isothiocyanato-3-oxapentyl)-2,3,4,6-tetra-O-acetyl-α-D-mannopyranoside (5). 4 (0.657 g, 4.478 mmol) and 4 Å sieves (6 g) were added to a solution of 3 (2.001 g, 4.058 mmol) dissolved in CH₂Cl₂ (50 ml). Under an argon atmosphere, BF₃·Et₂O (0.514 ml, 4.058 mmol) was slowly added and the reaction was stirred for 7 hours. Solid NaHCO₃ (1 g) was added to the reaction. The mixture was filtered over celite (CH₂Cl₂) and concentrated in vacuo. The resulting residue was purified by silica gel chromatography (1:1 Hexanes:EtOAc) to give 0.4174 g (24%) of highly viscous yellow oil. ¹H NMR (500 MHz, CDCl₃) δ 5.13-5.35 (m, 3H), 4.82 (s, 1H), 4.15-4.30 (m, 1H), 3.94-4.10 (m, 3H), 3.70-3.83 (m, 1H), 3.63 (bs, 6H), 2.09 (s, 3H), 1.98 (s, 3H), 1.92 (s, 3H) ppm. The ¹H NMR spectrum agreed with previously reported values⁸.
**Generation 4.0 PAMAM-based thiourea 1-O-(5-thiourea-3-oxapentyl)-α-D-mannopyranoside dendrimer (6).** A solution of G4 PAMAM dendrimer (0.6965g, 8.56 μmol) was dissolved in DMSO (5 mL) and combined with 5. The solution was stirred for 24 hours. The resulting solution was lyophilized to give an oily solid. The resulting solid was suspended in a 1:1 mixture of MeOH and H2O (20 mL) along with 1M NaOMe (100 μL) and was stirred overnight. The resulting solution was neutralized with amberlite and then filtered. MeOH was removed under reduced pressure, and the resulting aqueous solution was dialyzed against H2O (MW cutoff 1 kDa). The solution was lyophilized to give 0.18g (61% yield) of product. $^1$H NMR (500 MHz, CDCl$_3$) δ 7.98 (bs, 1H, amide NH’s), 7.79 (bs, 1H, amide NH’s), 7.48 (bs, 2H, CH$_2$NHC(S)NHCH$_2$), 4.72 (bs, 2H), 4.60 (bs, 2H), 4.46 (bs, 1H), 3.20-3.80 (m, 19H), 2.95-3.18 (m, 4H), 2.00-2.80 (m, 8H) ppm. The $^1$H NMR spectrum agreed with previously reported values$^9$. 
Attempted generation 4.0 PAMAM-based thiourea 1-O-(5-thiourea-3-oxapentyl)-6-(1-hexadecyl-1-azonia-4-azabicyclo[2.2.2]octane)-α-D-mannopyranoside dendrimer (7) Method 1: Compound 6 (50 mg, 1.47 μmol) was dissolved in DMF (1.5 mL) and cooled to 0°C. TsCl (16.9 mg, 88.64 μmol) was added with 2,6-DTBP (18.29 mg, 21 μL, 88.64 μmol). The reaction was allowed to warm to RT and was stirred for 2 h. DMF was removed at reduced pressure leaving a white fluffy solid. This solid was dissolved in acetonitrile, cooled to 0°C, and C_{16}DABCO (16.5 mg, 48.8 μmol) was added. The reaction proceeded over night. The resulting compound was concentrated in vacuo.

Method 2: Compound 6 (50 mg, 1.47 μmol) was dissolved in pyridine (1.45 mL) and cooled to 0°C while the reaction continued to stir. The reaction stirred for 2 h at RT. The reaction was again cooled to 0°C. C_{16}DABCO was added and the reaction was allowed to stir over night at RT. The resulting solution was dialyzed against H_{2}O (MW cutoff 1 kDa). The solid left behind was transferred to the dialysis tubing by dissolving it in CH_{2}Cl_{2}. The resulting retentate was lyophilized.
Method 3: The compound 6 (50 mg, 1.47 µmol) was dissolved in pyridine (1.46mL) and CH₂Cl₂ (1 mL), cooled to 0°C making sure the reaction was stirring. The reaction was stirred at RT for 2 h. Afterwards, the reaction was again cooled to 0°C. C₁₆DABCO was added and the reaction was allowed to stir over night at RT. The resulting solution was dialyzed against H₂O (MW cutoff 1 kDa). The solid left behind was transferred to the dialysis tubing by dissolving it in H₂O. After dialysis, the retentate was placed on the lyophilizer.
VI. Acronyms and Abbreviations

C_{16}DABCO or 1,4-diazabicyclo[2.2.2]octane
PAMAM or Poly(amido amine) dendrimer
In(OTf)_{3} or Indium triflate
NaOMe or Sodium Methoxide
EtOAc or Ethyl Acetate
MeOH or Methanol
DMF or Dimethylformamide
DMSO or Dimethyl sulfoxide
Literature Cited


