Total Syntheses of Conjugation-Ready Repeating Units of Acinetobacter baumannii AB5075 for Glycoconjugate Vaccine Development

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Abstract: Acinetobacter baumannii is an opportunistic pathogen that causes serious nosocomial infections. One of the multidrug-resistant strains, AB5075, can result in bacteremia, pneumonia and wound infections associated with high morbidity and mortality. The structurally unique glycans on the surface of these bacteria are attractive targets for the development of glycoconjugate vaccines. Here, we report the first total synthesis of the densely functionalized trisaccharide repeating unit of A. baumannii AB5075 as well as two analogues. The construction of 1,2-cis linkages between the rare sugars relies on a double-serial inversion strategy. The judicious selection of building blocks and reaction conditions allowed for stereoselective glycosylations, the installation of acetamido groups and the (S)-3-hydroxybutanoyl chain.

Introduction

Infections caused by multidrug-resistant bacteria are increasing in frequency and result in high morbidity and mortality.[1–4] Acinetobacter baumannii, a Gram-negative coccobacillus, is one of the most prevalent causes of nosocomial infections,[5,6] and is responsible for severe urinary tract infections, bacteremia and pneumonia.[7,8] The high adaptability of this bacterium has rendered multiple strains of A. baumannii resistant to almost all antimicrobials,[9–12] such that the World Health Organization lists this bacterium in the highest category of pathogens posing an imminent threat to human health. Vaccines against A. baumannii pathogens are urgently needed.

The outer membrane of A. baumannii AB5075 is surrounded by high molecular weight capsular polysaccharides (CPS)[13] that form a discrete layer on the bacterial surface, that assists in evasion of the host immune defenses and increases antibiotic tolerance.[14–16] CPS can trigger a specific immune response,[17,18] and renders the polysaccharides ideal targets for development of glycoconjugate vaccines.[19,20]

Identification of the immunogenic epitope is the key step for the development of novel vaccines.[21] Antigen candidates synthesized based on CPS are important tools to elucidate the structures of anti-CPS antibodies.[22,23] Here, we report the first syntheses of a series of conjugation-ready oligosaccharides related to the CPS repeating unit of AB5075 as the basis for further immunological studies.

Results and Discussion

The CPS of Acinetobacter baumannii AB5075 consists of two linear trisaccharide repeating units [−−3]-β-d-ManpNAcA-(1→4)-β-d-ManpNAcA-(1→3)-α-L-GuloN-acetamido groups and the (S)-3-hydroxybutanoyl acetyl in a ratio of approximately 2.5:1 (Figure 1A).[23] The repeating units bear N-acetyl groups on D-mannuronic acid and a (S)-3-hydroxybutanoyl group on D-bacillosamine. Three 1,2-cis linkages including two challenging β-mannosides and a terminal α-glycosidic linkage, together with the presence of a (S)-3-hydroxybutanoyl group and dense N-acetyl groups make the trisaccharides very challenging targets to synthesize.

The retrosynthesis of target repeating unit RU-1 reveals that trisaccharide 5 can be converted to the desired molecule via reduction, acetylation of azide groups and global deprotection (Figure 1B). The transformation of 4 to 5 involves the key reaction in this work: levulinoyl (Lev) groups are removed before double-serial inversion creates the two 1,2-cis mannoside linkages. Trisaccharide skeleton 4 can be obtained by [1+1] and [1+2] glycosylations, the β-selectivity could be ascertained by the neighboring participation of 2-O-Lev groups.[24]

The total synthesis commenced with the preparation of the orthogonally protected rare sugar building blocks (Scheme 1). D-Bacillosamine derivative 1 was synthesized starting from the α-selective glycosylation of selenoglycoside 6[25] with aminopropyl linker 7 using NIS/TMSOTf as a promoter to give α-linked glycoside 8 in 85% yield.[26] The linker is designed in anticipation of the conjugation to carrier protein or a microarray surface.[27] Removal of the 4,6-silylde group employing
HF·pyridine afforded diol 9, then the C6 hydroxyl in 9 was tosyalted using 4-toluenesulfonfyl chloride to form 10 in 73 % yield, followed by treatment with sodium iodide in refluxing acetone to obtain iodide 11. Subsequently, reduction with tributyltin hydride and azobisisobutyronitrile (AIBN) at 75 °C gave 12 in 87 % yield. The transformation of d-fucosamine derivative 12 to d-bacillosamine derivative 13 was carried out using nucleophilic displacement of the triflate. First, the C4 hydroxyl in 12 was triflated using triflic anhydride (Tf₂O) and pyridine to form a 4-O-triflate intermediate. After a brief extraction, the triflate was treated with sodium azide in DMF to yield the desired 13. [28] Removal of 2-naphthylmethyl (Nap) protecting group provided building block 1 in 89 % yield.

For the synthesis of glucuronic acid building block 2, the 4,6-benzylidene group of known thioglycoside 14 was cleaved to form diol 15 (Scheme 1). Selective oxidation of the C6 hydroxyl group in 15 using 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO) and bis(acetoxy)iodobenzene (BAIB) afforded the C6 carboxylic acid that was esterified with methyl iodide and K₂CO₃ to give 16 in 62 % yield over two steps. [30] Introduction of the fluorenylmethoxycarbonyl (Fmoc) protecting group on the C4 hydroxyl furnished desired building block 2 in good yield. As for the synthesis of building block 3, oxidation and esterification of 17 gave glucuronate 18 in 68 % yield. Then, cleavage of the Nap ether in 18 and subsequent replacement by a Lev ester group furnished building block 3.

With the building blocks in hand, the initial attempt to prepare trisaccharide started with the union of monosaccharides 1 with 2 in the presence of NIS/TfOH promoter, to form the desired disaccharide (Scheme 2). The triethylamine quenched also cleaved the Fmoc group to furnish β-linked disaccharide 20.
in 67% yield while none of the \(\alpha\)-isomer was observed. The stereoselectivity is the result of the participating Lev ester in \(2\). Similarly, the \([1 + 2]\) glycosylation of disaccharide \(20\) with building block \(3\) catalyzed by NIS and TFOH yielded the trisaccharide \(4\) with complete \(\beta\)-selectivity. The cleavage of Lev esters was followed by the conversion of the hydroxyl groups in \(21\) into triflates using \(\text{Tf}_2\text{O}\) and pyridine, concomitant replacement with tetrabutylammonium azide (TBAN\(_3\)) in the axial positions gave the desired azide \(5\) in 72% yield. The four azide groups were then reduced with zinc followed by acetylation with acetic anhydride (Ac\(_2\)O) in THF to obtain trisaccharide \(22\). However, the attempted hydrolysis of the methyl ester with lithium hydroxide failed to produce the desired compound \(23\) as the very fragile glycosidic bond between two \(\delta\)-mannuronic acids was cleaved in the aqueous basic environment to generate mono- and disaccharide fragments.

Mindful of the lability of glycosidic bond that required us to avoid treating the trisaccharide with strong base, the C6 carboxylic acids in D-glucurionate building blocks were protected as benzyl instead of methyl esters. These benzyl ethers would be cleaved during final hydrogenolysis.\(^{[32]}\) Similar to the synthesis of compound \(2\), efficient oxidation and subsequent esterification with benzyl bromide and NaHCO\(_3\) led to the D-glucuronate \(24\) in 66% yield as a benzyl ester (Scheme 3A). Fmoc protection of the C4 hydroxyl group completed the synthesis of building block \(25\). Selective cleavage of 4,6-benzylidene in thioglycoside \(26\)\(^{[33]}\) formed \(27\) in 75% yield. Diol \(27\) was then converted to benzyl glucuronate \(28\) by regioselective oxidation of the C6-OH and esterification. Levulinoylation of the C2 hydroxyl group in \(28\) using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC·HCl) and DMAP produced building block \(29\).

Scheme 2. Attempted assembly of trisaccharide RU-1.

Scheme 3. A) Optimized \(\delta\)-glucuronate building blocks \(25\) and \(29\); B) Assembly of trisaccharide repeating unit RU-1.
Following the initial route to RU-1, two sequential glycosylations using NIS/TfOH as promoter produced trisaccharide backbone 31 with exclusive β-selectivity (Scheme 3B). Cleavage of the Lev esters with hydrazine acetate freed two hydroxyl groups that were converted to 2',2''-bis triflates followed by replacement with azide nucleophiles in the axial position to generate azide 33 in good yield. The four azide groups were reduced with zinc in the presence of acetic acid followed by acetylation with acetic anhydride in THF. Finally, the N-acetyl sugar was subjected to hydrogenolysis after extraction to furnish the repeating unit RU-1 in 35% yield over two steps.

With RU-1 in hand, the repeating unit of AB5075 containing a (S)-3-hydroxybutanoyl chain served as the next target. The (S)-3-hydroxybutanoyl group must be installed prior to inversion due to the density of azides after inversion (Figure 2). Thus, an orthogonal azide group has to be included in building block 34 for subsequent reduction and coupling. A trichloroacetamido (TCA) group was chosen to mask the amino group of C2 in 34. Glycosylations of building blocks 34, 25 and 35 will form trisaccharide backbone 36, the exclusive azide in 36 will be reduced and (S)-3-hydroxybutanoyl chain can be introduced of this stage. If successful, the following inversion and global deprotection will produce the desired target molecule RU-2.

The synthesis of 34 commenced with the reduction of 11 with tributyltin hydride and AIBN at 85 °C, to reduce the C2-azide in 11 to the corresponding amino group and give 39 in 90% yield (Scheme 4). Installation of a TCA group to protect the amine was followed by inversion at the C4 position, β-bacillosamine derivative 41 was obtained in high yield. Removal of Nap ether with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) prepared 34 for glycosylation. For building block 35, selective benzylidene opening of 42 using BH$_3$·THF and TMSOTf afforded diol 43 in 70% yield. Subsequent regioselective oxidation of the C6 hydroxyl group to the corresponding carboxylic acid was achieved efficiently using TEMPO/BAIB, followed by treatment with benzyl bromide and NaHCO$_3$ to furnish 44 in 59% overall yield. The synthesis of 35 was completed after Lev protection of the C2 hydroxyl in 87% yield.

With all building blocks in hand, the coupling of 34 and 25 to form disaccharide 45 was explored. The conditions established for the synthesis of RU-1, a NIS/TfOH catalyzed glycosylation, afforded disaccharide 45 in low yield (Table 1, Entry 1). Higher temperatures (0 °C and 25 °C) or a NIS/TMSOTf promoter system (Table 1, Entry 2, 3 and 4) did not greatly increase the

Figure 2. Retrosynthetic analysis of target molecule RU-2.

Scheme 4. Synthesis of building blocks 34 and 35.
yield. Possibly, introduction of the TCA group may be responsible for the decrease in the nucleophilicity of receptor 34.[36]

In order to improve the efficiency of the glycosylation to obtain disaccharide 45, the Fmoc protecting group in 25 was replaced with a strong electron-donating tert-butyldimethylsilyl ether (TBS) group. Glucuronate building block 46 was obtained after protection of the C4-OH as a TBS ether in 24 in 90% yield (Scheme 5A). The glycosylation of 34 using glycosylating agent 46 and NIS/TIOH as promoter at 0 °C was much more efficient and yielded 73% β-linked disaccharide 47 (Scheme 5B). Disaccharide acceptor 45 was furnished after TBS deprotection by TBAF and [1 + 2] glycosylation of 45 with 35 yielded trisaccharide 36 in β selectively. Chain elongation started from azide reduction of 36. The Staudinger reaction failed for this trisaccharide,[37] while treatment with 1,3-propanedithiol was too mild to reduce the azide, instead, one chlorine of the TCA group was cleaved to give a 2-dichloroacetamido-4-azido product.[34,38] Finally, treatment of 36 with excess zinc and acetic acid at 40 °C in THF successfully converted the azide to the amine, subsequent coupling with freshly made (S)-3-O-benzylbutyryl chloride[39,40] gave 37 in 60% yield over two steps. The Lev esters were cleaved to get diol 48 with two hydroxyl groups ready for inversion. However, triflation of the equatorial hydroxyl groups in 48 and its concomitant displacement with TBAN failed to form the desired 2',2''-bis azide product 38. The inversion succeeded only for the 2'-triflate, while the 2'-triflate was not substituted by azide but was hydrolyzed to give product 49 in 57% yield. The configuration and inversion site were determined with the assistance of HMBC and HSQC (Supporting information). Increasing the temperature to 80 °C resulted in glycosidic bond cleavage between the two uronates.

Double-serial inversion conditions worked efficiently on 32 but not 48 mainly due to the installation of the (S)-3-hydroxybutanoyl chain, the most important difference between these two diols. Possibly, the steric hindrance of this chain interfered with the nucleophilic attack of azide on the 2'-triflate in the axial positions (Scheme 5B).[28,41]

To access the desired trisaccharide 38, we turned to introduce the two axial azide groups by two separated inversions, that requires first inversion on disaccharide and second one after trisaccharide is assembled. Reduction of disaccharide 47 and subsequent coupling with (S)-3-O-benzylbutyryl chloride afforded 50 in 52% yield over two steps (Scheme 6). Lev ester was then removed and resulting alcohol

### Table 1. Glycosylation to synthesize disaccharide 45.

| Entry | Promoter     | Temperature [°C] | Result                                      |
|-------|--------------|------------------|---------------------------------------------|
| 1     | NIS/TfOH     | –20 to 0         | 24% product formed, acceptor recovered      |
| 2     | NIS/TfOH     | 0                | 25% product formed, acceptor recovered      |
| 3     | NIS/TMSOTf   | 0                | 19% product formed, acceptor recovered      |
| 4     | NIS/TfOH     | 0 to 25, overnight | 33% product formed, acceptor recovered      |

![Scheme 5. A) Synthesis of building block 46; B) Assembly of trisaccharide 49 and the attempted inversion.](image-url)
51 was employed to triflation and inversion, unfortunately, none of desired product 52 was observed but rather starting material 51 was recovered. Since the steric hinderance effect of \((S)\)-3-hydroxybutanoyl chain was so strong that separated inversions strategy did not give access to desired product, we tried to temporarily protect the amine with Fmoc and proceed chain elongation after inversion stage (Scheme 7). Trisaccharide 36 was reduced efficiently to an amine by zinc and protected with Fmoc group to give 53 in 43% overall yield. Delevulinoylation of 53 using hydrazine acetate furnished diol 54 that was subjected to triflation by TfO and pyridine. The subsequent inversion also failed to produce any azide product.

Attempts to synthesize the natural repeating unit RU-2 did not meet with success, as the functionalization of amine within the \(d\)-bacillosamine derivative hinders the inversion process by disfavoring the substitution from the axial position. However, it is still a significant move to investigate the indispensability of 2',2''-acetamide and 2''-acetamide with the trisaccharide intermediates 48 and 49 we obtained.

Thus, to better understand the important role played of \(N\)-acetyl groups in antibody recognition, two analogues related to RU-2 were synthesized (Scheme 8). RU-A1 was obtained after the global deprotection of 48 in 52% yield. Synthesis of analogue RU-A2 involved conversion of azide in 49 to the corresponding NHAc by treatment with zinc and \(Ac_2O\), followed by the hydrogenolysis catalyzed over Pd/C to afford the second analogue RU-A2 in 41% yield over two steps. Analogues RU-A1 and RU-A2, together with natural repeating unit RU-1, will be employed in glycan microarray to identify the key epitope that elicits specific immune responses against native ABS075 CPS.

**Conclusion**

We report the first total synthesis of a densely functionalized aminoglycoside trisaccharide repeating unit of \(A. baumannii\) ABS075 as well as two analogues containing a challenging \((S)\)-3-hydroxybutanoyl chain. Synthetic challenges associated with the complicated trisaccharide were overcome including \(\beta\)-mannoside synthesis, introduction of \((S)\)-3-hydroxybutanoyl and the incorporation of labile glycosidic bonds. Orthogonally protected rare sugar building blocks provided efficient and stereoselective synthetic access to the trisaccharide, \(S_2\) substitution of 2',2''-bis triflate allowed for the construction of multiple 1,2-cis linkages. Although the inversion on the trisaccharide containing the \((S)\)-3-hydroxybutanoyl chain failed to deliver the desired 2',2''-bis azide product, the analogues based on RU-2 provide the opportunity to investigate key epitopes that induce an antibody response against the native CPS of \(A. baumannii\). Double-serial inversion in the context of complex oligosaccharides is a novel approach to the synthesis of complex aminoglycosides. Conjugation-ready sugars carrying an aminopropyl linker allows for easy access to glycan microarrays and in vivo immunological evaluation, en route to the
development of a synthetic glycoconjugate vaccine against *A. baumannii*.

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**Conflict of Interest**

The authors declare no conflict of interest.

**Keywords:** amides · carbohydrates · oligosaccharides · total synthesis · vaccines

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**Scheme 8.** Synthesis of the analogues RU-A1 and RU-A2.
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