Total Synthesis of an All-1,2-cis-Linked Repeating Unit from the Acinetobacter baumannii D78 Capsular Polysaccharide

Dancan K. Njeri and Justin R. Ragains*

ABSTRACT: Chemical synthetic efforts have resulted in the preparation of the assigned tetrasaccharide repeating subunit from the Acinetobacter baumannii KL4-associated capsular polysaccharide. A convergent synthetic strategy hinging on a 1,2-cis-selective [2+2] glycosylation to generate the fully protected tetrasaccharide was key to the success of this synthesis.

Acinetobacter baumannii is a Gram-negative, opportunistic bacterial pathogen associated with illness in individuals suffering from traumatic injury as well as the immunocompromised.1−5 It is one of the six nosocomial “ESKAPE” pathogens associated with drug resistance and virulence,6 and it has been deemed an urgent threat due to the prevalence of clinically relevant strains that are extensively drug-resistant (resistant to at least one agent in all but one or two categories of antimicrobials) and even pandrug-resistant (resistant to all approved antimicrobials).7,8 Despite a multitude of efforts, a vaccine remains elusive.9,10 Meanwhile, A. baumannii is associated with a substantial number of capsular polysaccharide (CPS),11 lipooligosaccharide,12 and O-glycan structures13 that might prove to be promising candidates for semisynthetic glycoconjugate vaccine development.14,15

As part of a research program aimed at synthesizing glycans associated with the cell surface of A. baumannii, we became interested in the KL4 (CPS biosynthetic gene cluster)-associated repeating unit 1 depicted in Scheme 1. Originally isolated by Kenyon et al.16 from multidrug-resistant A. baumannii strain D78 and assigned using a combination of chemical and spectroscopic analysis, the repeating unit of the KL4 CPS has an intriguing structure. It consists of N-acetyl-D-quinovosamine (QuiNAc), N-acetyl-D-galactosaminuronic acid (GalNAcA), N-acetyl-D-galactosamine (GalNAc), and the 4,6-pyruvate ketal of N-acetyl-D-galactosamine (Pyr-GalNAc), a frequently occurring motif in microorganisms.17 Particularly striking is the fact that all glycosidic linkages are of the 1,2-cis/α configuration that is synthetically more challenging than 1,2-trans/β linkages.18,19 Establishing the 1 → 4 glycosidic linkage between GalNAc and GalNAcA in reasonable yield appeared to be the greatest challenge. In this work, we recount our efforts that have led to the successful synthesis of tetrasaccharide repeating unit 1. Particularly noteworthy are two 1,2-cis-selective O-glycosylation reactions as well as our resorting to a convergent [2+2] synthetic approach when our initial efforts toward a linear synthesis gave substandard results.

Our initial retrosynthesis (Scheme 1) consisted of disconnecting 1 to 2-azido-2-deoxygalactose donor 2 (which we believed to be a dramatically simplifying common intermediate toward GalNAcA, GalNAc, and Pyr-GalNAc) and 2-azido-2-deoxyglucose donor 3. Steric hindrance due to
the bulky di-tert-butylsilylene (DTBS) protecting group in 2 could ensure 1,2-cis selectivity in the relevant O-glycosylations.20 Meanwhile, the 1,2-cis-selective O-glycosylation leading to the linkage between 3 and linker molecule 4 (which would enable eventual conjugation to carrier proteins for, e.g., vaccine development14,15 or conjugation to a glycan array21) could be carried out according to a previously developed synthetic strategy.22

Our synthesis of the QuiNAc-linker molecule portion 8 (Scheme 2) commenced with known benzylidene-protected 2-azido-2-deoxythioglucoside 5 (synthesized in five steps from D-glucosamine).23 Walking benzylidene to position 4 was effected with BH3·THF/TMSOTf followed by acetylation to generate 6. Subsequent oxidative hydrolysis of thioglycoside (NBS, H2O, and acetone) and conversion of intermediate lactol to trichloroacetimidate (CCl3CN and K2CO3)24 furnished donor 3. We then performed multiple attempts at O-glycosylation of linker 4 with 3 according to conditions previously reported by Boons and co-workers (TMSOTf, excess of thiophene, and low temperature).22 While yields were reasonable, selectivity was modest [<5:1 in favor of 1,2-cis relative to an unwanted byproduct that we attribute to the 1,2-trans isomer (data not shown)]. We attribute this to the very high reactivity of 4 resulting in modest selectivity. Coming off our recent success25 (and noting the successes of others26) in the development of 1,2-cis-selective glucosylation using glucosyl imidates and a combination of either triflic acid or TMSOTf in 1,4-dioxane, we performed glycosylation of 4 with 3 using 1,4-dioxane as the solvent under dilute conditions at room temperature (~18 °C) (Scheme 2). This furnished target glycoside 7 in 75% yield with only traces of the observable undesired byproduct. Four additional steps of manipulation (Scheme 2; methanalysis, tosylation, Finkelstein iodination, and ionic reduction with NaCNBH3 in diethylene glycol diethyl ether) resulted in the formation of alcohol 8, which was ready for further manipulation.

Having reached the incipient phase of tetrasaccharide assembly (Scheme 3), we reacted alcohol 8 with DTBS-protected N-phenyltrifluoroacetimidate 2 (prepared in six steps from triacetyl D-galactal)27 in the presence of triflic acid (HOTf) to provide a high yield of disaccharide 9 as the only observed isomer. This is likely due to the bulk of DTBS that deflects “top-side” attack by the acceptor.20 Subsequent manipulation of 9 [DTBS removal with HF-pyridine, two-step oxidation to uronic acid,28 and methylation with TMSCHN229 (Scheme 3)] resulted in alcohol 10. While the potential low reactivity of this acceptor (due to the axial disposition of the C-4 alcohol and electron-withdrawing effects from an azido at position 2 and a -CO2Me at position 6) was of concern, this potential flaw may have ultimately been to our advantage (Scheme 4, vide infra). In any case, subsequent glycosylation of 10 with 2 (HOTf and CH2Cl2) resulted in a 79% yield of trisaccharide 11 as the only observable isomer, suggesting that the potential low reactivity of 10 was not fatal to the synthesis. Pleased with this result, we removed DTBS (HF-pyridine) and attempted glycosylation of the resulting 11, once again, with donor 2. To our great surprise and dismay, these attempts at selective glycosylation at position 6 of the nonreducing-end diol of 12 with 2 resulted in low yields and complex mixtures of products of apparent unselective and even double glycosylation. The cause of such low-yielding reactions with poor regioselectivity is mysterious to us at present.

### Scheme 2. Synthesis of the QuiNAc Portion

| Step | Reaction | Conditions | Yield (%) |
|------|----------|------------|-----------|
| 1 | BH3·THF, TMSOTf, CH2Cl2, rt. | 71% |
| 2 | Ac2O, DMAP, Et3N, CH2Cl2, rt. | 71% |
| 3 | NBS, acetone/H2O (10:1), rt., 87% |
| 4 | Cl3CCN, K2CO3, CH2Cl2, rt. | 90% |
| 5 | NaOAc, MeOH, CH2Cl2, rt. | 83% |
| 6 | TeCN, Py, CH2Cl2, rt. | 92% |
| 7 | NaI, 2-butanone, reflux |
| 8 | NaCNBH3, Ph(OH)2CH2Cl, HOEt, 120 °C | 84% (2 steps) |

### Scheme 3. Initial Assembly of a GalNAc/GalNAcA/ QuiNAc Trisaccharide

| Step | Reaction | Conditions | Yield (%) |
|------|----------|------------|-----------|
| 1 | BF3·Py, 0 °C | 73% |
| 2 | Ph(OAc)2, TEMPO, 0 °C | 71% |
| 3 | NaClO4, NaH2PO4, BuOH, l-acetylene, H2O, rt. |
| 4 | TMSCHN2, MeOH, rt. | 85%, 3-steps |
| 5 | NaOAc, MeOH, CH2Cl2, rt. | 84% |
| 6 | NBS, acetone/H2O, rt. | 86% |

### Scheme 4. Synthesis of a Pyr-GalNAc/GalNAc Disaccharide Donor

| Step | Reaction | Conditions | Yield (%) |
|------|----------|------------|-----------|
| 1 | BF3·Py, 0 °C | 73% |
| 2 | BF3·OEt2, CH2CN, rt. | 61% |
At this stage, we considered a number of alternatives, including benzylidation and subsequent “walking to the 4” [as with 5 → 6 (Scheme 2)] of diol 12, but we were dissatisfied with the attendant sacrifice of synthetic efficiency. Therefore, we devised a convergent approach that, while not being without its own risks, would avoid the intermediacy of 12 and streamline the synthesis. Synthesis of a suitable Pyr-GalNAc/GalNAc portion of 1 (Scheme 4) commenced with the glycosylation of 13 (prepared in seven steps from D-galactosamine) with 2 in the presence of HOTf to generate 14 with 1,2-cis as the only observed configuration at the newly forged linkage. Interestingly, this process resulted in epimerization of reducing-end thioglycoside. Regardless of this complication, separation of thioglycoside epimers at this stage was facile. Subsequent DTBS removal (HF-pyridine) preceded pyruvate ketal installation under equilibrating conditions (BF₃·Et,O) to furnish the thermodynamic ketal stereochemistry (15), which was confirmed through analysis of NMR chemical shifts. Oxidative hydrolysis of thioglycoside (NBS, H₂O, and acetone) and conversion to N-phenyl-trifluoroacetimidate provided disaccharide donor 16, which was ready for coupling to acceptor 10.

Because donor 16 lacked the highly efficacious DTBS group that practically ensures 1,2-cis stereochemistry, we approached the subsequent glycosylation with some trepidation (Scheme 5).

Scheme 5. Final Approach to the Target Tetrasaccharide

Treatment of a mixture of donor 16 and acceptor 10 in CH₂Cl₂ with TMSOTf at 18 °C resulted, to our delight, in a high yield of the desired, fully protected tetrascarachide 17 with 1,2-cis stereochemistry at the newly forged 1 → 4 linkage. In some instances (e.g., running the reaction at 0 °C), we could observe a minor product with a 13C signal appearing slightly above 100 ppm, suggesting that some of the undesired 1,2-trans isomer might be generated in small quantities. However, we were never able to isolate this byproduct in pure form. The 1,2-cis selectivity of this glycosylation may be attributable to the low reactivity of acceptor 10 and equilibration of anomeric triflates derived from 16 with the equatorial triflate (or an ion pair derived from it) being more reactive than the axial triflate as has been suggested by Codeé and co-workers. Blocking of “top-side attack” of 10 by the axial 4-position benzylxoy group in 16 may also be a factor.

With fully protected 17 in hand, conversion of azides, hydrolysis of methyl esters, and removal of benzyl protecting groups remained. Thus, treatment with thiourea acid in pyridine over a period of 80 h resulted in reduction of azides and acylation to the four acetamido groups in the final product. Subsequent hydrolysis of methyl esters (NaOH, MeOH, and THF) and hydrogenolysis of benzyl groups [H₂ and Pd(OH)₂] resulted in final product 18, the linker-attached monomer of 1. This synthesis proceeded in a total of 35 steps from commercially available starting materials and a longest linear sequence of 23 steps from D-glucosamine. NMR of the final product ([H, 13C, 13C-APT, COSY, HSQC, HMBC, and HOAHAA) as well as HRMS helped confirm the structure.

While differences were seen upon comparison of our spectra with those of the CPS originally characterized by Kenyon et al., two important points deserve mention. (1) Our product bears a linker, which represents a substantial perturbation of the original structure. (2) While Kenyon et al. do not report on any secondary structure associated with the original CPS, secondary structure would be expected to perturb the appearance of an NMR spectrum relative to a segment with a short chain length. The tetrasaccharide that we have prepared is necessarily devoid of secondary structure due to its short chain length. Due to the regiochemical reliability of procedures such as benzylidine walking [5 → 6 (Scheme 2)] and in the generation of known compound 13 (Scheme 4), primary alcohol oxidation to carboxylic acid using TEMPO/PhIO(OAc)₂ followed by Pinnick oxidation [9 → 10 (Scheme 3)], and the stereochemical verifiability and reliability of pyruvate ketal installation under equilibrating conditions [14 → 15 (Scheme 4)], we have high confidence in the structural assignment for 18. In addition to this, one-dimensional and two-dimensional (2D) NMR analysis assisted us in identifying critical HMBC correlations in 18, including the following: (1) between linker CH₂-O signals centered at ~3.71 and ~3.91 ppm and the QuiNAc anomeric carbon at 96.98 ppm as well as between the QuiNAc anomeric proton at 4.98 ppm and the linker CH₂-O 13C signal at 68.00 ppm, (2) between the GalNAc anomeric proton at 5.52 ppm and the QuiNAc C3 carbon at 79.20 ppm, and (3) between the GalNAc C4 proton at 4.58 ppm and the GalNAc anomeric carbon at 99.01 ppm. This accounts for three of four linkages, with the fourth linkage (1 → 6 linkage between PyrGalNAc and GalNAc) being harder to analyze at the stage of product 18 due to substantial signal overlap between these subunits. Nevertheless, this linkage was established with a high degree of confidence from a known set of precursors [13 and 2 (Scheme 4)] to establish 1,2-cis stereochemistry unambiguously as could be ascertained easily with 13C spectra of 14. Also noteworthy is the fact that all anomeric carbons of final product 18 appear at chemical shifts of <100 ppm, affirming that 1,2-cis stereochemistry has been established at all four of the glycosidic linkages. Thus, the expected stereochemical and regiochemical outcomes of key transformations in the synthesis of 18 are corroborated by 2D NMR data.

In conclusion, we have synthesized the assigned KL4-associated tetrascarachide repeating CPS subunit of A. baumannii D78 with a longest linear sequence of 23 steps. Especially noteworthy with this synthesis was the establishment of the glycosidic linkage between the linker and QuiNAc using dilute conditions in 1,4-dioxane and a convergent [2+2]
glycosylation to establish the fully protected tetrasaccharide banking on the low reactivity of acceptor 10. Additional efforts toward the synthesis of Acinetobacter baumannii cell-surface-associated glycans are underway and will be reported in due course.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.orglett.2c01034.

Experimental procedures, characterization data, and 1H, 13C, and 2D NMR spectra (PDF)

■ AUTHOR INFORMATION

Corresponding Author

Justin R. Ragains – Department of Chemistry, Louisiana State University, Baton Rouge, Louisiana 70806, United States; orcid.org/0000-0002-2521-5396; Email: jragains@lsu.edu

Author

Dancan K. Njeri – Department of Chemistry, Louisiana State University, Baton Rouge, Louisiana 70806, United States

Complete contact information is available at: https://pubs.acs.org/doi/10.1021/acs.orglett.2c01034

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The authors acknowledge the National Science Foundation (CHE-1665208 and CHE-2101153) for generous support of this research. The authors thank Ms. Connie David [Louisiana State University (LSU)] for assistance with high-resolution mass spectrometry. The authors also thank Dr. Thomas Weldeleighor (LSU) for assistance with, and helpful conversations about, NMR.

■ REFERENCES

(1) Huang, W.; Yao, Y.; Long, Q.; Yang, X.; Sun, W.; Liu, C.; Jin, X.; Li, Y.; Chu, X.; Chen, B.; Ma, Y. Immunization against Multidrug-Resistant Acinetobacter baumannii Effectively Protects Mice in Both Pneumonia and Sepsis Models. PLoS One 2014, 9, No. e100727.

(2) García-Quintanilla, M.; Pulido, M. R.; McConnell, M. J. First Steps Towards a Vaccine against Acinetobacter baumannii. Curr. Pharm. Biotechnol. 2014, 14, 897–902.

(3) Lazareanu, V.; Porosnicu, M.; Gandac, C.; Moisil, T.; Baditoiu, Al.; Lazza, R.; Musta, V.; Crisan, A.; Marinescu, A.-R. Infection with Acinetobacter baumannii in an Intensive Care Unit in the Western Part of Romania. BMC Infect. Dis. 2016, 16 (Suppl.), 95–100.

(4) Vila, J.; Pachón, J. Therapeutic Options for Acinetobacter baumannii Infections. Expert Opin. Pharmacother. 2008, 9, 587–599.

(5) Wisplinghoff, H.; Edmond, M. B.; Pfaffer, M. A.; Jones, R. N.; Wenzel, R. P.; Seifert, H. Acinetobacter Species in United States Hospitals: Clinical Features, Molecular Epidemiology, and Antimicrobial Susceptibility. Clin. Infect. Dis. 2000, 31, 690–697.

(6) De Oliveira, D. M. P.; Forde, B. M.; Kidd, T. J.; Harris, P. N. A.; Schembri, M. A.; Beatson, S. A.; Paterson, D. L.; Walker, M. J. Antimicrobial Resistance in ESKEAPE Pathogens. Clin. Microbiol. Rev. 2020, 33, No. e00181-19.

(7) Luo, G.; Lin, L.; Ibrahim, A. S.; Baquir, B.; Pantapalangkoor, P.; Bonomo, R. A.; Doi, Y.; Adams, M. A.; Russo, T. A.; Spellberg, B. Active and Passive Immunization Protects against Lethal, Extreme Drug Resistant-Acinetobacter baumannii Infection. PLoS One 2012, 7, No. e29446.

(8) Dijkshoorn, L.; Nemec, A.; Seifert, H. An Increasing Threat in Hospitals: Multidrug-Resistant Acinetobacter baumannii. Nat. Rev. Microbiol. 2007, 5, 939–951.

(9) Pachon, J.; McConnell, M. J. Considerations for the Development of a Prophylactic Vaccine for Acinetobacter baumannii. Vaccine 2014, 32, 2534–2536.

(10) Gellings, P. S.; Wilkins, A. A.; Morici, L. A. Recent Advances in the Pursuit of an Effective Acinetobacter baumannii Vaccine. Pathogens 2020, 9, 1066.

(11) Giguère, D. Surface Polysaccharides from Acinetobacter baumannii: Structures and Syntheses. Carbohydr. Res. 2015, 418, 29–43.

(12) Vinogradov, E. V.; Duus, J.O.; Brade, H.; Holst, O. The Structure of the Carbohydrate Backbone of the Lipopolysaccharide from Acinetobacter baumannii Strain ATCC 19606. Eur. J. Biochem. 2002, 269, 422–430.

(13) Iwashikw, J. A.; Seper, A.; Weber, B. S.; Scott, N. E.; Vinogradov, E.; Stratil, C.; Reiz, B.; Cordwell, S. J.; Whittal, R.; Schild, S.; Feldman, M. F. Identification of a General O-Linked Protein Glycosylation System in Acinetobacter baumannii and Its Role in Virulence and Biofilm Formation. PLoS Pathog. 2012, 8, No. e1002758.

(14) Adamo, R.; Niño, A.; Castagner, B.; Bouteireau, O.; Berti, F.; Bernardes, G. J. L. Synthetically Derived Glycoprotein Vaccines: Current Status and Future Directions. Chem. Sci. 2013, 4, 2995–3008.

(15) Anish, C.; Schumann, B.; Pereira, C. L.; Seeberger, P. H. Chemical Biology Approaches to Designing Defined Carbohydrate Vaccines. Chem. Biol. 2014, 21, 38–50.

(16) Kenyon, J. J.; Speciale, I.; Hall, R. M.; De Castro, C. Structure of Repeating Unit of the Capsular Polysaccharide from Acinetobacter baumannii D78 and Assignment of the K4 Gene Cluster. Carbohydr. Res. 2016, 434, 12–17.

(17) Hager, F. J.; Sutlzl, L.; Stefanovic, C.; Blaukopf, M.; Schaffer, C. Pyruvate Substitutions on Glycoconjugates. Int. J. Mol. Sci. 2019, 20, 4929.

(18) Nigudkar, S. S.; Demchenko, A. V. Stereocentrally Controlled 1,2-cis Glycosylation as the Driving Force of Progress in Synthetic Carbohydrate Chemistry. Chem. Sci. 2015, 6, 2687–2704.

(19) Demchenko, A. V. Stereoselective Chemical 1,2-cis-Glycosylation: From ‘Sugar Ray’ to Modern Techniques of the 21st Century. Expert Opin. Ther. Targets 2003, 2003, 1225–1240.

(20) Imamura, A.; Ando, H.; Kurogi, S.; Tanabe, G.; Muraoka, O.; Ishida, H.; Kiso, M. Di-tert-butylsilylene (DTBS) Group-Directed α-Selective Galactosylation Unaffected by C-2 Participating Functionalities. Tetrahedron Lett. 2003, 44, 6725–6728.

(21) Horlacher, T.; Seeberger, P. H. Carbohydrate Arrays as Tools for Research and Diagnostics. Chem. Soc. Rev. 2008, 37, 1414–1422.

(22) Park, J.; Kawatkar, S.; Kim, J.-H.; Boons, G.-J. Stereoselective Glycosylations of 2-Azido-2-deoxy-glucosides Using Intermediate Pyruvate Substitutions. Tetrahedron 2007, 63, 8185–8197.

(23) Chang, C. W.; Lin, M. H.; Chan, C. K.; Su, K. Y.; Wu, C. H.; Lo, W. C.; Lam, S.; Cheng, Y. T.; Liao, P. H.; Wong, C. H.; Wang, C. Automated Quantification of Hydroxyl Reactivities: Prediction of Glycosylation Reactions. Angew. Chem., Int. Ed. 2021, 60, 12413–12415.

(24) Schmidt, R. R.; Jung, K. H. Trichloroacetimidates. In Carbohydrates in Chemistry and Biology; Ernst, B., Hart, G. W., Sinn, P., Eds.; Wiley-VCH: Weinheim, Germany, 2000; Vol. 1, pp 5–59.

(25) Njeri, D. K.; Pertuit, C. J.; Ragains, J. R. 1,2-cis-Selective Glucosylation Enabled by Halogenated Benzyl Protecting Groups. Org. Biomol. Chem. 2020, 18, 2405–2409.

(26) Demchenko, A.; Stauch, T.; Boons, G.-J. Solvent and Other Effects on the Stereoselectivity of Thioglycoside Glycosidations. Synlett 1997, 1997, 818–820.

(27) Wang, L.; Zhang, Y.; Overkleeft, H. S.; van der Marel, G. A.; Codée, J. D. C. Reagent Controlled Glycosylations for the Assembly of Well-Defined Pel Oligosaccharides. J. Org. Chem. 2020, 85, 15872–15884.
(28) Hagen, B.; van Dijk, J. H. M.; Zhang, Q.; Overkleeft, H. S.; van der Marel, G. A.; Codée, J. D. C. Synthesis of the \textit{Staphylococcus aureus} Strain M Capsular Polysaccharide Repeating Unit. \textit{Org. Lett.} \textbf{2017}, \textit{19}, 2514−2517.

(29) Hashimoto, N.; Aoyama, T.; Shioiri, T. New Methods and Reagents in Organic Synthesis. 14. A Simple Efficient Preparation of Methyl Esters with Trimethylsilyldiazomethane (TMSCHN$_2$) and Its Application to Gas Chromatographic Analysis of Fatty Acids. \textit{Chem. Pharm. Bull.} \textbf{1981}, \textit{29}, 1475−1478.

(30) Kanemitsu, T.; Daikoku, S.; Kanie, O. Solid-Phase Synthesis of Sialyl Tn Antigen. \textit{J. Carbohydr. Chem.} \textbf{2006}, \textit{25}, 361.

(31) van der Vorm, S.; Hansen, T.; Overkleeft, H. S.; van der Marel, G. A.; Codée, J. D. C. The Influence of Acceptor Nucleophilicity on the Glycosylation Reaction Mechanism. \textit{Chem. Sci.} \textbf{2017}, \textit{8}, 1867−1875.