

Semi-Synthetic Glycoconjugate Vaccine Lead Against *Acinetobacter baumannii* 17978

Julinton Sianturi, Patricia Priegue, Jing Hu, Jian Yin, and Peter H. Seeberger*

Abstract: *Acinetobacter baumannii* is a opportunistic bacterial pathogen responsible for serious nosocomial infections that is becoming increasingly resistant against antibiotics. Capsular polysaccharides (CPS) that cover *A. baumannii* are a major virulence factor that play an important role in pathogenesis, are used to assign serotypes and provide the basis for vaccine development. Synthetic oligosaccharides resembling the CPS of *A. baumannii* 17978 were printed onto microarray slides and used to screen sera from patients infected with *A. baumannii* as well as a monoclonal mouse antibody (mAb C8). A synthetic oligosaccharide emerged from glycan array screening as lead for the development of a vaccine against *A. baumannii* 17978. Tetrasaccharide **20** is a key epitope for recognition by an antibody and is a vaccine lead.

Introduction

Acinetobacter baumannii (*A. baumannii*), a Gram-negative bacterium, is a common cause of soft tissue and urinary tract infections, septicemia, pneumonia and meningitis.^[1] Untreated infections are associated with a high burden of morbidity and mortality rates as high as 50%.^[2] Resistant *A. baumannii* are becoming a global concern due to the inefficiency of antibiotics of last resort. The World Health Organization now lists *A. baumannii* as a critical target in urgent need of new antimicrobials.^[3]

Glycoconjugate vaccines are a powerful means to prevent infectious diseases.^[4] *A. baumannii* produces capsular polysaccharides (CPS) that surround the outer membrane.^[5] CPS, consisting of repeating units of oligosaccharides (K units), are a major virulence factor that protects bacteria against the environment helping them to evade the host immune response. CPS covering pathogens differ from mammalian glycans such that autoimmunity or allergies in humans are unlikely to be triggered. To date, the structures of twenty *A. baumannii* CPS have been elucidated^[6] among the more than 90 serotypes.^[7] *A. baumannii* ATCC17978 (K3 CPS type) CPS consists of a core trisaccharide repeating unit ([\rightarrow 3)- α -D-Gal(1 \rightarrow 6)- β -D-Glc(1 \rightarrow 3)- β -D-GalNAc(1 \rightarrow)] β -linked to a tri-acetylated 2,3-diamino-D-glucuronate at 4-OH of Gal and β -linked GlcNAc at 6-OH of Gal as branches. The core trisaccharide fragment is also found in the CPS of several other strains such as *A. baumannii* ATCC NIPH146,^[8] 17961,^[9] SMAL,^[10] and LUH5537 (KL22 and PSgc9).^[6,11] Monoclonal antibodies raised against isolated *A. baumannii* ATCC17978 CPS provided 55% protection and the antibody reacted with 62% of *A. baumannii* clinical isolates of different clones (342/554 strain).^[12] The abundant expression of amino sugars such as Quip4NAc (*N*-acetyl-D-quinovosamine), tri-acetylated glucuronic acid and Pse5Ac7Ac (pseudaminic acid) on CPS of *A. baumannii* isolates,^[2,9,13] suggests that such amino sugars play an important role in the pathogenicity of these bacteria^[14] (Figure 1).

Isolated native CPSs are very complex and produced inconclusive immunological results.^[15] Synthetic oligosaccharide antigens resembling CPS epitopes have emerged as an attractive option to isolated CPS since well-defined chemical structures avoid impurities and such glycoconjugates have been proven effective in protecting animals and humans from bacterial infections.^[16] Here, we report the first synthesis of well-defined oligosaccharides resembling the *A. baumannii* ATCC17978 CPS. The synthetic glycans were printed onto microarray slides and screened against the blood of patients in an effort to identify a lead antigen for the development of a semi-synthetic glycoconjugate vaccine against this *A. baumannii* strain.

Results and Discussion

The *A. baumannii* 17978 pentasaccharide repeating unit contains five different monosaccharides (Figure 2A) and presents several synthetic challenges including access to the tri-acetylated glucuronic acid unit and the efficient installa-

[*] Dr. J. Sianturi, P. Priegue, Prof. Dr. P. H. Seeberger
 Department of Biomolecular Systems, Max-Planck Institute of Colloids and Interfaces
 Am Mühlenberg 1, 14476 Potsdam (Germany)
 E-mail: peter.seeberger@mpikg.mpg.de

P. Priegue, Prof. Dr. P. H. Seeberger
 Department of Chemistry and Biochemistry, Freie Universität Berlin
 Arnimallee 22, 14195 Berlin (Germany)

J. Hu, Prof. Dr. J. Yin
 Wuxi School of Medicine, Jiangnan University
 Lihu Ave. 1800, 214122 Wuxi (China)

© 2022 The Authors. Angewandte Chemie International Edition published by Wiley-VCH GmbH. This is an open access article under the terms of the Creative Commons Attribution Non-Commercial NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

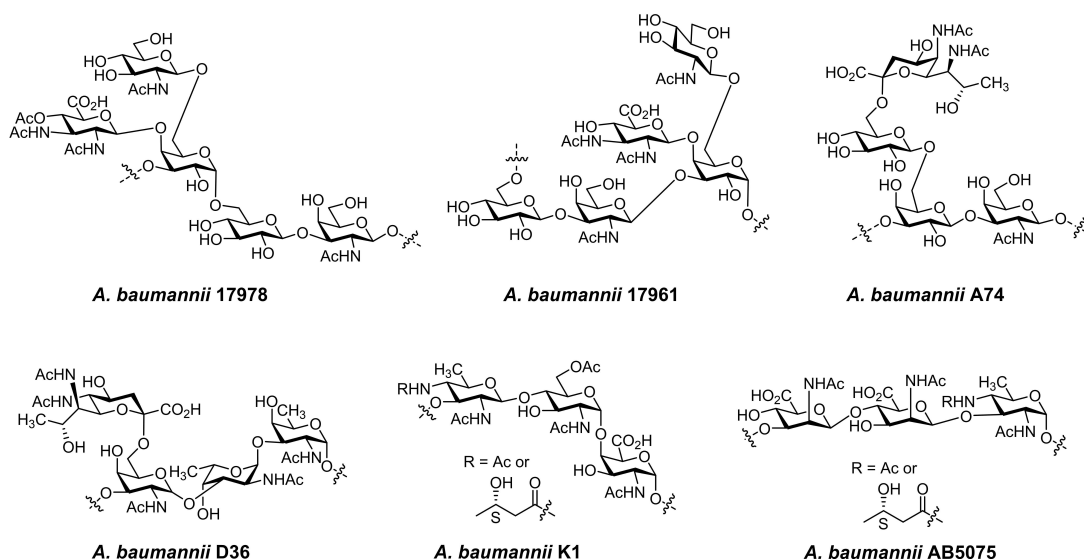


Figure 1. *A. baumannii* CPS repeating units contain rare amino sugars.

tion of diamino-D-glucuronate **12** building block that carries multiple electron withdrawing protecting groups. The minimal epitope of the *A. baumannii* 17978 repeat unit remains to be established using synthetic oligosaccharides. Sixteen mono- and oligosaccharides (**15–30**) resembling the repeating unit of *A. baumannii* 17978 were designed to enable detailed glycan microarray studies (Figure 2B). Four pentasaccharide fragments were targeted, including pentasaccharide **15**, spanning the entire length of the repeating unit and pentasaccharides **16–18** resemble the entire molecule including side-chain modifications of the diamino-D-glucuronate. Deletion sequence glycans **19–30** are designed to help to define key epitope and to better understand the role of the acetyl groups in antibody binding, antigens without an acetyl group at the tri-acetylated glucuronic acid fragment (**16**, **21** and **23**) were also synthesized. All synthetic glycans contain an aminopentyl linker at the reducing end for microarray printing and protein conjugation.

Synthesis of protected monosaccharides **3–14** relied on slightly modified literature precedence (Supporting Information, Schemes S1 and S2),^[17] while a synthesis of diamino-D-glucuronate **12** from commercially available D-glucosamine was developed. The installation of azide group in **12** relied on double inversion at C3 via Lattrell-Dax inversion^[17g,h] (Supporting Information, Scheme S3).

The synthesis of pentasaccharides **15–18** as well as deletion sequences via a linear approach used building blocks **3–14** (Figure 2A). The union of galactosamine **3** with *N*-(benzyl)benzyloxycarbonylaminopentanol (aminopentanol linker), followed by cleavage of the Fmoc protecting group provided exclusively β -linked product **31** (Figure 3A). Glycosylation with **9**,^[17e] followed by fluorenylmethoxycarbonyl (Fmoc) cleavage afforded disaccharide **32**. Global deprotection of **32** using lithium hydroxide followed by hydrogenation afforded **29** in 60% yield over two steps. Placement of α -galactose employed di-(*tert*-butyl)silylene

acetal^[18] at the C4 and C6 position of galactose **7** enabled straightforward handling and excellent reactivity. Glycosylation in the presence of *N*-iodosuccinimide (NIS) and trimethylsilyl trifluoromethanesulfonate (TMSOTf) proceeded exclusively in α -fashion to furnish trisaccharide **33** ($^1J_{\text{C,H}} = 172.1$ Hz, Supporting Information). An acetyl group on the diamino-D-glucuronate unit, suggests that exchanging electron withdrawing benzoyl ester groups on glucose for electron donating protecting groups is the key to constructing the desired pentasaccharide **15**. Debenzoylation of **33** with sodium methoxide freed a poorly nucleophilic hydroxyl group that could not be protected as using 2-naphthylmethyl (Nap), benzyl (Bn) and Fmoc groups. Sterically hindered nucleophiles in some oligosaccharides may fail to react in fluorenylmethoxycarbonylation and Williamson ether syntheses.^[19] Cleavage of the silyl group in **34** followed by hydrogenation afforded trisaccharide deletion sequence **25**.

To overcome the poor nucleophilicity of trisaccharide **34** (Figure 3A), the hydroxyl group was reacted at the disaccharide stage. Galactose **31** was glycosylated with donor **10** to afford protected disaccharide **39**. Hydrolysis of the 2-*O*-benzoyl ester gave the free hydroxyl group before benzyl ether formation afforded the desired compound **40** in 40% yield accompanied by dibenzylated product **41** (21%). Regioselective ring opening of benzylidene acetal in **40** using borane tetrahydrofuran complex catalyzed with TMSOTf afforded **42**. Disaccharide **42** was glycosylated with **7**, followed by cleavage of the silyl group using pyridinium poly(hydrogen fluoride)^[20] (HF-Py) which provided **44**. Trisaccharide **44** bearing two hydroxyl groups was glycosylated with thioglycoside **4** to furnish **45**. The more reactive and accessible C6 primary alcohol of trisaccharide diol **44** results in selective formation of tetrasaccharide **45**. Global deprotection by hydrogenolysis using palladium on carbon (Pd/C) in EtOAc/*t*-BuOH/H₂O provided tetrasaccharide **19** in 57% yield. Finally, the diamino-D-glucuronate building

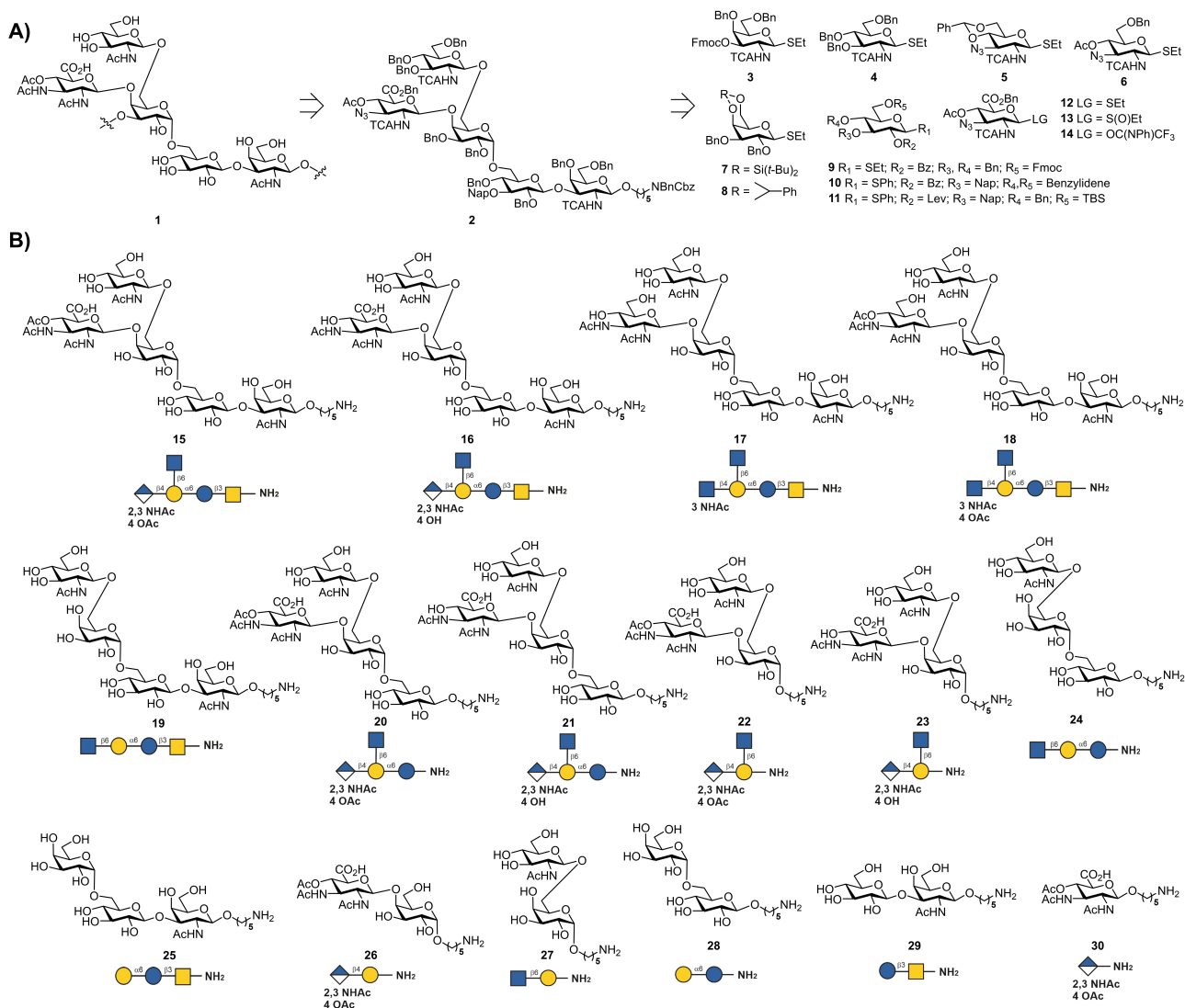


Figure 2. A) Repeating unit of *A. baumannii* 17978 CPS and related oligosaccharides synthesized from building blocks 3–14; B) Structure of *A. baumannii* 17978 related glycans 15–30. TCA = trichloroacetyl; N₃ = azide; Ac = acetyl; Bn = benzyl, Bz = benzoyl, TBS = *tert*-butyldimethylsilyl, Nap = 2-naphthylmethyl; Lev = levulinyl; Fmoc = fluorenylmethyloxycarbonyl; Cbz = carboxybenzyl; Ph = phenyl.

block was employed to glycosylate acceptor **45**. Glycosylation using **12** promoted by NIS and triflic acid at 0 °C for 2 h did not proceed to completion, but additional promoters and stirring at room temperature for 2 h gave the desired pentasaccharide **2** in poor yield (17%) and partial recovery of acceptor **45** (47% based on recovered starting material (b.r.s.m.)). A second attempt using oxidized diamino-D-glucuronate donor **13** activated by 2,4,6-tri-*tert*-butylpyrimidine and triflic anhydride provided trace product and separation using silica column chromatography was difficult. Schmidt diamino-D-glucuronate donor **14** promoted by NIS and TMSOTf afforded only 15% of **2** together with an unknown by-product. Low donor reactivity dramatically affected the outcome of the glycosylation as benzyl esters present in **12–14** decreased their reactivity.^[16b,17c] Since these conditions proved ineffective, we investigated more efficient route to the pentasaccharide. With a sufficient amount of

protected **2** in hand, reduction of the azide to acetamide and concomitant reduction of the TCA groups was achieved using activated zinc powder in a Ac₂O/AcOH/THF mixture, followed by catalytic hydrogenation to give pentasaccharide **15** in 34% over two steps (Figure 3B).

An efficient synthesis of pentasaccharide **15** had to address two crucial stages, namely replacement of the benzoyl group on glucose and the poor reactivity of diamino-D-glucuronates **12–14**. The C4 hydroxyl group of tetrasaccharide acceptor **45** is a poor nucleophile that renders this molecule a synthetic challenge. Instead of a benzoyl ester, a levulinoyl ester was employed that can be cleaved selectively in the presence of an acetyl group of triacetylated glucuronic acid fragment (Figure 4A). The conversion was carried out with the core of the pentasaccharide fragment. Subsequent union of **31** and donor **11** yielded β-linked disaccharide **46**, followed by cleavage of the TBS

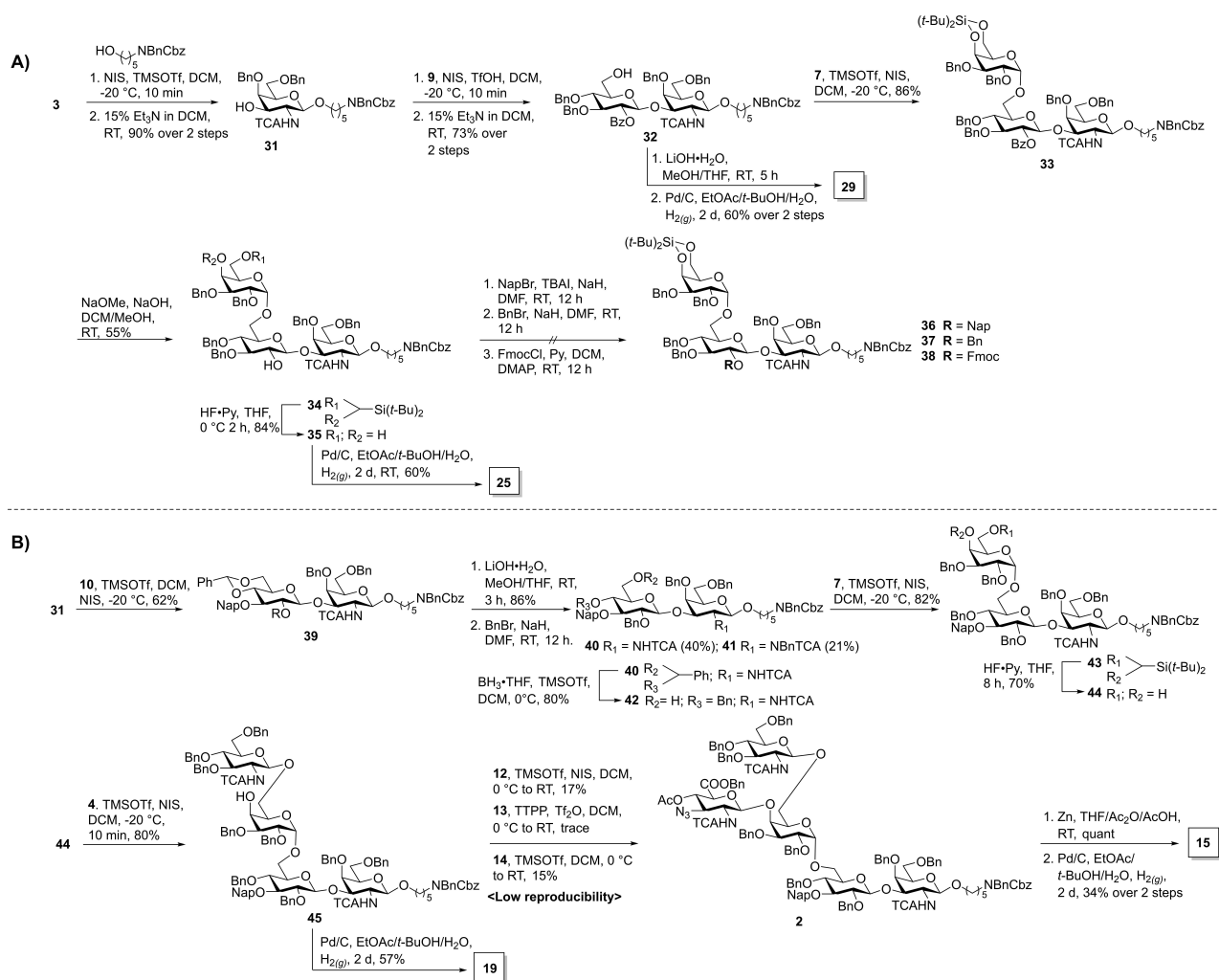


Figure 3. A) Synthesis of tri-, tetrasaccharides (**19**, **25**); B) Attempted synthesis of pentasaccharide **15**.

group to afford acceptor **47**. The disaccharide was glycosylated with building block **7** and treated with HF·Py to afford trisaccharide diol **49**, that in turn was glycosylated with **4** at the primary hydroxyl, before the resulting tetrasaccharide **50** was glycosylated, furnishing desired pentasaccharide **51** in 67% yield. These results revealed that replacing the C5 benzyl ester and C4 acetyl group of diamino-D-glucuronate donors with a benzylidene protecting group significantly improved the yield of the glycosylation. Target **52** was obtained by glycosylation of **50** and **6** in 53% yield with exclusive β -selectivity. Compounds **51** and **52** were treated with activated zinc and hydrazine acetate, followed by Pd/C catalyzed hydrogenolysis furnished pentasaccharide **17** (41%) as well as **18** (41%), respectively. Removal of the benzylidene group in **51** with aqueous acetic acid^[16b] (80%) at 55 °C delivered pentasaccharide diol in quantitative yield. Selective oxidation of the C6 hydroxyl group using TEMPO/BAIB and catalytic amounts of acetic acid, followed by protection of the acid moiety as benzyl ester and acetylation of the C4 hydroxyl group gave **53** in 65% over four steps. Zinc-mediated reduction of **53**, followed by ester cleavage and hydrogenolysis afforded oligosaccharides **15** and **16** in

42% and 28% yield over four steps, respectively. Using the Lev group in **11** and diamino glucosamine **5** building blocks ensured an efficient synthesis of oligosaccharides **15–16**. Although the modification of the core of pentasaccharide fragment is not ideal, this is the only way to overcome the issue of scalability and reproducibility of *A. baumannii* glycans **15** and **16**.

The synthesis of tetrasaccharides **20** and **21** followed a similar synthetic approach (Figure 4B). Reaction of thioglycosides **8** and **54** afforded disaccharide **55**. The $J_{C,H}$ coupling of 172.4 Hz clearly confirm the formation of α -galactosidic linkage (Supporting Information). Participation of the C2 benzoyl group reduces the reactivity of **54**, while ether protected glycosyl donor **8** is significantly more reactive.^[21] The disaccharide was next reacted with the aminopentyl linker to provide **56** in 80% yield and subjected to hydrolysis of the benzoyl ester to afford **57**. Hydrogenation of **57** provided disaccharide **28** in 55% yield. Naphthyl ether formation at the C2 position and hydrolysis of the benzylidene with (+)-camphor-10-sulfonic acid and ethane-1-thiol as an additive^[22] smoothly delivered **59** in excellent yield. Linking disaccharide diol **59** and thioglycoside **4**

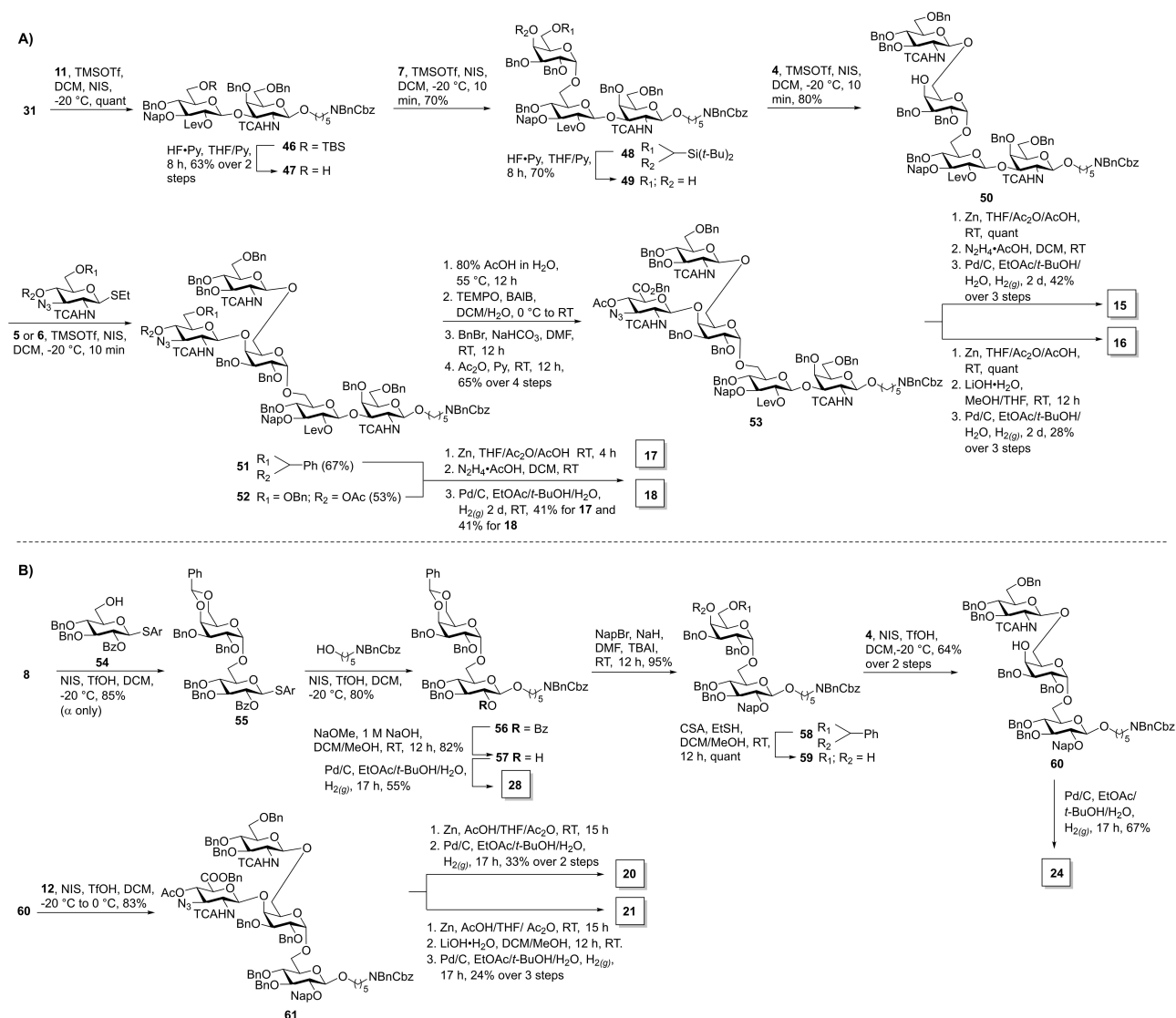


Figure 4. A) Efficient syntheses of pentasaccharides **15** and **16** using building blocks **5** and **11** followed by modification of the core of pentasaccharide fragment. B) Synthesis of tetrasaccharides **20** and **21** using direct glycosylation with diamino-D-glucuronate **12** building block.

provided **60** that was subjected to hydrogenolysis with Pd/C to give trisaccharide **24** in 67% yield. To our surprise, the glycosylation between **60** and diamino-D-glucuronate donor **12** provided desired tetrasaccharide **61** in excellent yield (83%). We concluded that the reactivity of the hydroxyl group at the C4-position of the tetrasaccharide acceptor is key to the installation of **12**. Unlike trisaccharide acceptor **60**, the C4 hydroxyl group in tetrasaccharide **45** is a poor nucleophile shows mainly due to steric factors. When non-oxidized building block **5** carrying a C4–C6 benzylidene acetal was used, the reaction was forced to completion. Azide reduction followed by global deprotection of **61** afforded oligosaccharide **20** (33% over two steps) and nonacetylated product **21** (24% over three steps).

Oligosaccharides **22** and **23** were assembled via a linear approach using monosaccharide building blocks **4**, **7**, **8**, **64**–**66**, and **11**. Installation of a spacer- α -linkage galactose at the reducing end proved challenging (Table S1, Supporting

Information). Donor **8** contains a 4,6-*O*-benzylidene acetal carrying ether protection at O2 and O3 that typically results in α -selectivity^[23] gave product **67** in poor selectivity as an inseparable mixture of isomers (70%, 1:2.5, α/β). Interestingly, coupling donor **8** with thioglycoside **54** without ether as solvent gave exclusively α -product **55** (Figure 4B). We concluded that the structural rigidity of the acceptor affected the selectivity and yield of the glycosylation.^[23] Changing benzyl ether protection to a Nap ether at O3, gave desired product with better α -selectivity (2.5:1, α/β). Surprisingly, removal of the O3 protecting group to free the hydroxyl or placement of allyl groups resulted in a better yield and α -selectivity **69** (89%, 4:1, α/β) as judged by NMR and **70** (96%, 4.9:1, α/β , isolated yield). To prepare the reducing end galactose in large amounts with an easy purification, 4,6-*O*-di-(*tert*-butyl)silylene acetal (**7**) resulted in desired product **71** in 72% yield exclusively as the α -isomer ($^1J_{\text{CH}}$ = 171.2 Hz, Supporting Information). After cleavage of the

silyl group, the hydroxyl at C6 was selectively glycosylated with **4** to afford disaccharide **73** (Figure 5A), then was subjected to hydrogenation to obtain disaccharide **27** in good yield (77%). As expected, the coupling between diamino-D-glucuronate donor **12** and disaccharide acceptor **73** smoothly provided trisaccharide **74** in 64% yield. Global deprotection via Zn-mediated reduction, ester hydrolysis and hydrogenolysis afforded oligosaccharides **22** (70% over two steps) and **23** (64% over three steps).

Assembly of deletion sequences **26** and **30** containing the tri-acetylated glucuronic acid fragment were carried out via a stepwise synthesis using galactose building blocks **68** and **12** (Figure 5B). α -Product **68** was subjected to regioselective ring opening of the benzylidene acetal using triethylsilane catalyzed by TMSOTf to liberate the free hydroxyl group at the C4 position that was glycosylated with **12** to obtain protected disaccharide **75** in 87% yield. Reduction using activated zinc and hydrogenation using Pd/C provided disaccharide **26** in 41% yield over two steps. For the synthesis of spacer linked tri-acetylated glucuronic acid, glycosylation of the linker with **12** yielded exclusively monosaccharide **76** (68%) that was subjected to global deprotection via Zn-mediated reduction followed by hydrogenolysis to furnish desired monosaccharide **30** in 39% yield over two steps.

Glycan arrays are useful tools to identify minimal glycan epitopes of synthetic sugars by screening human sera.^[24] Synthetic glycans **15–30** and unrelated oligosaccharides were printed onto glass slides (Figure 6A, Figure S1, Supporting Information). Sera from infected patients and 007sp reference sera, as control, were screened for antibodies binding to the glycans resembling cell CPS of *A. baumannii* ATCC17978.^[25]

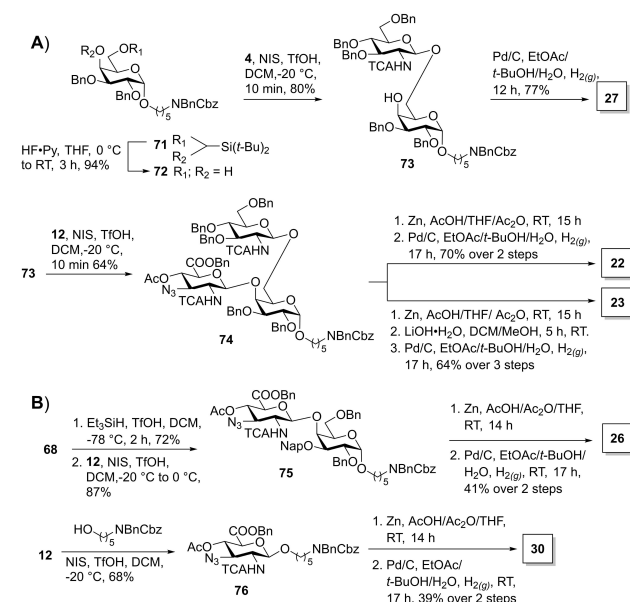


Figure 5. Assembly of short oligosaccharides. A) Synthesis of trisaccharides **22** and **23**. B) Synthesis of disaccharide **26** and monosaccharide **30**.

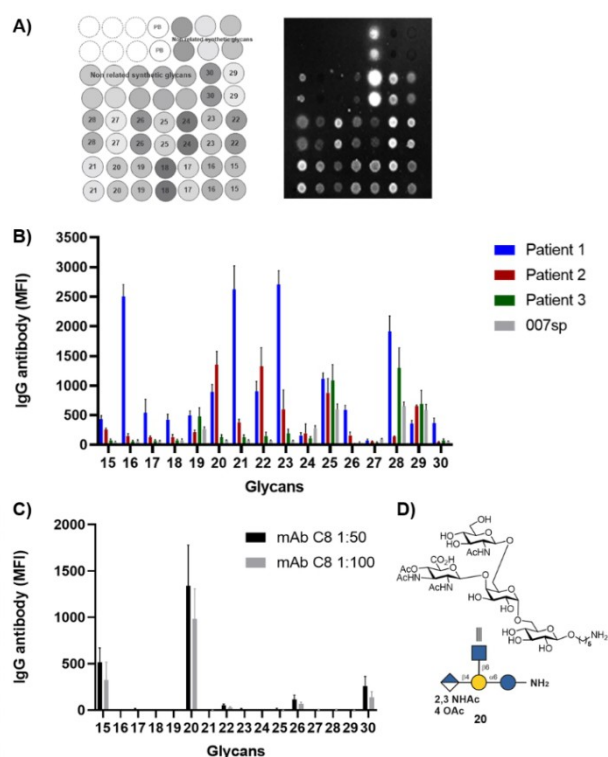


Figure 6. Glycan array analysis of *A. baumannii* ATCC17978 oligosaccharides. A) Printing pattern of microarray and binding pattern of human serum to immobilized glycans. B) IgG antibody binding to glycans with sera from infected patients. A serum dilution of 1:100 was used. C) IgG antibody binding to glycans with the monoclonal mouse antibody C8. Dilutions of 1:50 and 1:100 of mAb C8 were used. MFI, mean fluorescence in intensity (mean \pm standard deviation); PB, printing buffer; For glycan structures please see Figure 2B. D) Structure of the identified repeating unit.

Sera from infected patients contain antibodies against the bacterium that recognize the oligosaccharides resembling the native CPS structure.^[16c] The IgG binding pattern is complex but oligosaccharides **16**, **20–23** and **28** showed the highest signal (Figure 6B). Tetrasaccharides **20** or **21** may be considered the minimum glycotopes as they elicit the highest intensities. Three serum samples are not sufficient to determine whether or not acetylation plays an important role in binding. One patient may have been infected by another pathogenic strain with a CPS that contains a similar structure. Compared to the reference sera 007sp, oligosaccharides **15**, **18**, **24**, **27**, **30** are specifically recognized by sera from patients infected with *A. baumannii* but not by sera from vaccinated persons and almost no immune cross-reactivity with *S. pneumoniae* is observed.

Additional sera from a different source were screened (Figure S2, Supporting Information). In total, nine samples from patients infected with unknown *A. baumannii* strains were tested. Not all sera recognized the oligosaccharides (**21–23** showed no signal) probably because these patients were infected with different strains or did not develop antibodies against the bacterium. Antibodies bound to oligosaccharide **20** and to unrelated oligosaccharides (**25–**

29). Oligosaccharides **25–29** may represent motives present in the CPS or LPS of other bacteria and cause cross-reactivity and/or unspecific binding.^[26]

The glycans were screened with the monoclonal mouse antibody C8 that is specific for the strains HUMC1 and ATCC17978.^[27] The mAb specifically binds the acetylated oligosaccharides and tetrasaccharide **20**. In addition, C8 also bound to shorter oligosaccharides (**22**, **26** and **30**) and to pentasaccharide **15**, but not to the non-acetylated oligosaccharides (Figure 6C). These results clearly show that 2,3-diacetamido-4-*O*-acetyl glucopyranosyl uronate plays an important role for antibody binding. The mAb bound strongly to oligosaccharide **20**, such that we can conclude that tetrasaccharide **20** is a vaccine lead (Figure 6D). In order to further investigate the role of acetylation, oligosaccharides **20** and **21** will be tested in vivo as a next step to develop a protective vaccine against *A. baumannii* infection. Structures similar to oligosaccharide **20** are also present in other strains^[28] and a potential vaccine could protect against infections with other *A. baumannii* strains via a cross-reactive immune response.

Conclusion

We describe the first total syntheses of 16 oligosaccharides resembling the capsular polysaccharide of *A. baumannii* 17978. The installation of the diamino-D-glucuronate fragment relied on the reactivity of the nucleophile. The glycosylation between non-oxidized building block **5** carrying a C4–C6 benzylidene acetal and poor glycosyl acceptor **45** yielded desired pentasaccharide **51**. The synthetic glycans were printed on microarray slides and screened with the sera of infected patients and a monoclonal antibody (mAb C8). Tetrasaccharide **20** is well recognized by antibodies and constitutes a potential vaccine candidate. The 2,3-diacetamido-4-*O*-acetyl glucopyranosyl uronate plays an important role for immunological evaluation. A glycoconjugate based on **20** will be tested in *A. baumannii* challenge studies in a mouse model.

Acknowledgements

We gratefully acknowledge the Max-Planck Society for generous financial support. We thank Prof. Bettina Fries (Stony Brook University NY, USA) for providing us serum samples. We also thank Dr. Brad Spellberg and Dr. Brian Luna (USC Keck School of Medicine, Los Angeles, USA) for providing the monoclonal antibody C8. Open Access funding enabled and organized by Projekt DEAL.

Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available in the Supporting Information of this article.

Keywords: *Acinetobacter Baumannii* · Carbohydrate · Glycans · Immunology · Total Synthesis

- [1] N. C. Gordon, D. W. Wareham, *Int. J. Antimicrob. Agents* **2010**, *35*, 219–226.
- [2] R. G. Lees-Miller, J. A. Iwashkiw, N. E. Scott, A. Seper, E. Vinogradov, S. Schild, M. F. Feldman, *Mol. Microbiol.* **2013**, *89*, 816–830.
- [3] a) CDC, Antibiotic Resistance Threats in the United State. **2019**, 64–68. <https://www.cdc.gov/drugresistance/pdf/threats-report/2019-ar-threats-report-508.pdf>; b) HHS Food and Drug Administration, *Fed. Regist.* **2014**, *79*, 32464–32481; c) E. Tacconelli, et al., *Lancet Infect. Dis.* **2018**, *18*, 318–327.
- [4] a) O. Yaqub, S. Castle-Clarke, N. Sevdalis, J. Chataway, *Soc. Sci. Med.* **2014**, *112*, 1–11; b) M. García-Quintanilla, M. R. Pulido, R. López-Rojas, J. Pachón, M. J. McConnell, *Trends Microbiol.* **2013**, *21*, 157–163; c) R. D. Astronomo, D. R. Burton, *Nat. Rev. Drug Discovery* **2010**, *9*, 308–324.
- [5] T. A. Russo, N. R. Luke, J. M. Beanan, R. Olson, S. L. Sauberan, U. MacDonald, L. W. Schultz, T. C. Umland, A. A. Campagnari, *Infect. Immun.* **2010**, *78*, 3993–4000.
- [6] D. Giguère, *Carbohydr. Res.* **2015**, *418*, 29–43.
- [7] a) D. Hu, B. Liu, L. Dijkshoorn, L. Wang, P. R. Reeves, *PLoS One* **2013**, *8*, e70329; b) J. J. Kenyon, R. M. Hall, *PLoS One* **2013**, *8*, e62160.
- [8] N. P. Arbatsky, M. M. Shneider, J. J. Kenyon, A. S. Shashkov, A. V. Popova, K. A. Miroshnikov, N. V. Volozhantsev, Y. A. Knirel, *Carbohydr. Res.* **2015**, *413*, 12–15.
- [9] L. L. MacLean, M. B. Perry, W. Chen, E. Vinogradov, *Carbohydr. Res.* **2009**, *344*, 474–478.
- [10] E. Fregolino, V. Gargiulo, R. Lanzetta, M. Parrilli, O. Holst, C. De Castro, *Carbohydr. Res.* **2011**, *346*, 973–977.
- [11] A. S. Shashkov, J. J. Kenyon, N. P. Arbatsky, M. M. Shneider, A. V. Popova, K. A. Miroshnikov, R. M. Hall, Y. A. Knirel, *Carbohydr. Res.* **2016**, *435*, 173–179.
- [12] F.-L. Yang, T.-C. Lou, S.-C. Kuo, W.-L. Wu, J. Chern, Y.-T. Lee, S.-T. Chen, W. Zou, N.-T. Lin, S.-H. Wu, *Vaccine* **2017**, *35*, 1440–1447.
- [13] J. J. Kenyon, A. M. Marzaioli, R. M. Hall, C. De Castro, *Glycobiology* **2014**, *24*, 554–563.
- [14] a) I. M. Lee, F.-L. Yang, T.-L. Chen, K.-S. Liao, C.-T. Ren, N.-T. Lin, Y.-P. Chang, C.-Y. Wu, S.-H. Wu, *J. Am. Chem. Soc.* **2018**, *140*, 8639–8643; b) R. Wei, X. Yang, H. Liu, T. Wei, S. Chen, X. Li, *ACS Cent. Sci.* **2021**, *7*, 1535–1542.
- [15] J. J. Kenyon, A. M. Marzaioli, R. M. Hall, C. De Castro, *Glycobiology* **2015**, *25*, 881–887.
- [16] a) B. M. S. Seco, F.-F. Xu, A. Grafmüller, N. Kottari, C. L. Pereira, P. H. Seeberger, *ACS Chem. Biol.* **2020**, *15*, 2395–2405; b) S. Zhang, M. Sella, J. Sianturi, P. Priegue, D. Shen, P. H. Seeberger, *Angew. Chem. Int. Ed.* **2021**, *60*, 14679–14692; *Angew. Chem.* **2021**, *133*, 14800–14813; c) P. H. Seeberger, *Chem. Rev.* **2021**, *121*, 3598–3626.
- [17] a) H. S. Hahm, C.-F. Liang, C.-H. Lai, R. J. Fair, F. Schuhmacher, P. H. Seeberger, *J. Org. Chem.* **2016**, *81*, 5866–5877; b) M. Tsutsui, J. Sianturi, S. Masui, K. Tokunaga, Y. Manabe, K. Fukase, *Eur. J. Org. Chem.* **2020**, 1802–1810; c) P. I. Abronina, N. N. Malysheva, V. V. Litvinenko, A. I. Zinin, N. G. Kolotyorkina, L. O. Kononov, *Org. Lett.* **2018**, *20*, 6051–6054; d) N. Verma, Z. Tu, M.-S. Lu, S.-H. Liu, S. Renata, R. Phang, P.-K. Liu, B. Ghosh, C.-H. Lin, *J. Org. Chem.* **2021**, *86*,

- 892–916; e) Y. Yu, A. Kononov, M. Delbianco, P. H. Seeberger, *Chem. Eur. J.* **2018**, *24*, 6075–6078; f) A.-R. de Jong, B. Hagen, V. van der Ark, H. S. Overkleeft, J. D. C. Codée, G. A. Van der Marel, *J. Org. Chem.* **2012**, *77*, 108–125; g) C. Qin, B. Schumann, X. Zou, C. L. Pereira, G. Tian, J. Hu, P. H. Seeberger, J. Yin, *J. Am. Chem. Soc.* **2018**, *140*, 3120–3127; h) R. K. Singh, J. Sianturi, P. H. Seeberger, *Org. Lett.* **2022**, *24*, 2371–2375.
- [18] a) A. Imamura, A. Kimura, H. Ando, H. Ishida, M. Kiso, *Chem. Eur. J.* **2006**, *12*, 8862–8870; b) A. Imamura, H. Ando, S. Korogi, G. Tanabe, O. Muraoka, H. Ishida, M. Kiso, *Tetrahedron Lett.* **2003**, *44*, 6725–6728.
- [19] a) Z. Zhou, N. E. Behnke, L. Kürti, *Org. Lett.* **2018**, *20*, 5452–5456; b) K. Shibatomi, M. Kotozaki, N. Sasaki, I. Fujisawa, S. Iwasa, *Chem. Eur. J.* **2015**, *21*, 14095–14098.
- [20] G. A. Olah, J. T. Welch, Y. D. Vankar, M. Nojima, I. Kerekes, J. A. Olah, *J. Org. Chem.* **1979**, *44*, 3872–3881.
- [21] Z. Zhang, I. R. Ollmann, X.-S. Ye, R. Wischnat, T. Baasov, C.-H. Wong, *J. Am. Chem. Soc.* **1999**, *121*, 734–753.
- [22] Y. Liu, J. Zeng, J. Sun, L. Cai, Y. Zhao, J. Fang, B. Hu, P. Shu, L. Meng, Q. Wan, *Org. Chem. Front.* **2018**, *5*, 2427–2431.
- [23] M. Moumé-Pymbock, T. Furukawa, S. Mondal, D. Crich, *J. Am. Chem. Soc.* **2013**, *135*, 14249–14255.
- [24] F. Broecker, P. H. Seeberger, *Small Molecule Microarrays*, Springer, Heidelberg, **2017**, pp. 227–240.
- [25] D. Goldblatt, B. D. Plikaytis, M. Akkoyunlu, J. Antonello, L. Ashton, M. Blake, R. Burton, R. Care, N. Durant, I. Feavers, P. Fernsten, F. Fievet, P. Giardina, K. Jansen, L. Katz, L. Kierstead, L. Lee, J. Lin, J. Maisonneuve, M. H. Nahm, J. Raab, S. Romero-Steiner, C. Rose, D. Schmidt, J. Stapleton, G. M. Carlone, *Clin. Vaccine Immunol.* **2011**, *18*, 1728–1736.
- [26] a) M. R. M. Hussain, M. Hassan, I. Afzal, A. Afzal, *Egypt. J. Med. Hum. Genet.* **2012**, *13*, 1–9; b) T. D. Mubaiwa, E. A. Semchenko, L. E. Hartley-Tassell, C. J. Day, M. P. Jennings, K. L. Seib, *Pathog. Dis.* **2017**, *75*:ftx063.
- [27] T. B. Nielsen, P. Pantapalangkoor, B. M. Luna, K. W. Bruhn, J. Yan, K. Dekitani, S. Hsieh, B. Yeshoua, B. Pascual, E. Vinogradov, K. M. Hujer, T. N. Domitrovic, R. A. Bonomo, T. A. Russo, M. Lesczyniecka, T. Schneider, B. Spellberg, *J. Infect. Dis.* **2017**, *216*, 489–501.
- [28] a) C.-R. Lee, J. H. Lee, M. Park, K. S. Park, I. K. Bae, Y. B. Kim, C.-J. Cha, B. C. Jeong, S. H. Lee, *Front. Cell. Infect. Microbiol.* **2017**, *7*, 55; b) Y. Talyansky, T. B. Nielsen, J. Yan, U. Carlino-Macdonald, G. Di Venanzio, S. Chakravorty, A. Ulhaq, M. F. Feldman, T. A. Russo, E. Vinogradov, B. Luna, M. S. Wright, M. D. Adams, B. Spellberg, *PLoS Pathog.* **2021**, *17*, e1009291.

Manuscript received: June 30, 2022

Accepted manuscript online: August 11, 2022

Version of record online: September 1, 2022