Complete chemical structure of the K135 capsular polysaccharide produced by *Acinetobacter baumannii* RES-546 that contains 5,7-di-*N*-acetyl-8-epipseudaminic acid

Alexander S. Shashkov,[1] Anastasiya A. Kasimova,[1] Nikolay P. Arbatsky,[1] Sof'ya N. Senchenkova,[1] Andrei V. Perepelov,[1] Andrei S. Dmitrenok,[1] Alexander O. Chizhov,[1] Yuriy A. Knirel,[1] Mikhail M. Shneider,[2] Anastasia V. Popova,[3] Johanna J. Kenyon[*4]

[1]*N.D. Zelinsky Institute of Organic Chemistry, Russian Academy of Sciences, Moscow, Russia*

[2] *M.M. Shemyakin & Y. A Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, Moscow, Russia*

[3] *State Research Center for Applied Microbiology and Biotechnology, Obolensk, Moscow Region, Russia*

[4] *Centre for Immunology and Infection Control, School of Biomedical Sciences, Faculty of Health, Queensland University of Technology, Brisbane, Australia*

[*] Corresponding author. Tel.: +61 7 3138 2552; E-mail: johanna.kenyon@qut.edu.au

ABSTRACT

A structurally diverse capsular polysaccharide (CPS) in the outer cell envelope plays an important role in the virulence of the important bacterial pathogen, *Acinetobacter baumannii*. More than 75 different CPS structures have been determined for the species to date, and many CPSs include isomers of a higher sugar, namely 5,7-diamino-3,5,7,9-tetradeoxynon-2-ulosonic acid. Recently, a novel isomer having the D-*glycero*-L-*manno* configuration (5,7-di-*N*-acetyl-8-epipseudaminic acid; 8ePse5Ac7Ac) has been identified in the CPS from *A. baumannii* clinical isolate RES-546 [*Carbohydr. Res.* 513 (2022) 108531]. Here, the complete chemical structure of this CPS, designated K135, was elucidated. The CPS was found to have a branched tetrasaccharide K unit and to include the higher sugar as part of a 8ePse5Ac7Ac-(2→6)-α-Gal disaccharide branching from a →3)-α-D-Glc*p*NAc-(1→3)-β-D-Glc*p*NAc-(1→ main chain. Assignment of glycosyltransferases encoded by the CPS biosynthesis gene cluster in the RES-546 genome enabled the first sugar of the K unit, and hence the topology of the K135 CPS, to be determined.

Keywords*: Acinetobacter baumannii,* capsular polysaccharide, 5,7-diacetamido-3,5,7,9-tetradeoxynon-2-ulosonic acid; K135.

1. INTRODUCTION

Acinetobacter baumannii is an important human pathogen that has been implicated with serious multi-drug resistant Gram-negative bacterial infections worldwide [1, 2]. Promising alternative treatments to antimicrobials include monoclonal antibodies [3, 4] and bacteriophage cocktails [5] or their derivatives [6, 7], which both target specific structural epitopes of the capsular polysaccharide (CPS) that surrounds the bacterial cell surface. The presence of the CPS is also a major determinant of virulence [8], though recent evidence suggests that variations in the CPS structure also play an important role in pathogenesis [9].

While all strains studied to date produce a single type of CPS, the chemical structures of different types have been found to vary in the carbohydrate and non-carbohydrate content of the repeating oligosaccharides (K units) that form the CPS polymer [10-15]. The linkages between sugars and/or between K units can also vary [16-19]. To date, the structures for more than 75 *A. baumannii* CPS have been determined. Many of these include one of five different isomeric forms of a nine-carbon sugar, 5,7-diamino-3,5,7,9-tetradeoxynon-2-ulosonic acid. Recently, a sixth isomer, having the D-*glycero*-L-*manno* configuration (5,7-di-*N*-acetyl-8-epipseudaminic acid; 8ePse5Ac7Ac), not previously recovered from any biological source, has been identified in the CPS from *A. baumannii* clinical isolate RES-546 [20].

In this work, we report the complete structure of the *A. baumannii* RES-546 CPS that contains 8ePse5Ac7Ac. As *A. baumannii* CPS types are often designated 'K' names based on the type of CPS biosynthesis gene cluster present in the chromosome [21-23], the CPS type of the strain studied was named K135 following the finding of the KL135 gene cluster in the RES-546 whole genome sequence.

2. RESULTS

2.1. Monosaccharide analyses of the CPS recovered from RES-546

CPS was isolated from cells of *A. baumannii* RES-546 by extraction with phosphate buffered saline, and purified by Sephadex G-50 Superfine gel chromatography. Sugar analysis by GLC of the alditol acetates revealed the presence of galactose (Gal) and glucosamine (GlcN).

The absolute D configuration of these monosaccharides was established by the following glycosylation shifts, which were determined by a comparison with the published data of the corresponding non-substituted monosaccharides [24]: i) –2.1 and 4.6 ppm for C-5 and C-6, respectively, of the α-GlcNAc residue caused by substitution at position 6 with the α-Gal residue (70.7–72.8 = –2.1; 66.5–61.9 = 4.6); ii) 5.6 and –2.0 ppm for C-3 and C-4, respectively, of the β-GlcNAc residue caused by substitution at position 3 with the α-GlcNAc residue (80.7–75.1 = 5.6; 69.2–71.2 = –2.0); iii) 8.1 and 0.6 ppm for C-3 and C-4, respectively, of the α-GlcNAc residue caused by substitution at position 3 with the β-GlcNAc residue (80.1–72.0 = 8.1; 72.0–71.4 = 0.6).

Earlier, it has been shown that the CPS also contains a 5,7-diamino-3,5,7,9-tetradeoxynon-2-ulosonic acid identified as the 8-epimer of pseudaminic acid (8ePse) having the D-*glycero*-L-*manno* configuration [20].

The presence of signals for *N*-acetyl groups in the NMR spectra (δ_C 23.0-23.8 (CH_3) and 175.5–175.8 (CO), δ_H 2.00-2.10) indicated that the amino sugars are N-acetylated (for the ^{13}C NMR spectrum of the CPS see Fig. 1).

2.2. Resolution of the CPS structure

The structure of the CPS was established by NMR spectroscopy using a set of shift-correlated two-dimensional NMR experiments (^1H,^1H COSY, ^1H,^1H TOCSY, ^1H,^1H ROESY, ^1H,^{13}C

HSQC, and $^{1}H,^{13}C$ HMBC). The chemical shifts of the monosaccharide are tabulated in Table 1 and Table 2 and the ^{13}C NMR spectrum of the CPS is shown in Fig. 1. Based on typical ^{3}J coupling constants for the ring protons, the spin-systems were revealed for the constituent monosaccharides, including one α-Gal, one α-GlcNAc, and one β-GlcNAc residues, all being in the pyranose form. The chemical shift for C-5 (δ 76.7) confirmed that GlcNAc **A** is β-linked whereas those of 70.7 and 72.0 indicated that GlcNAc **B** and Gal **C** are α-linked.

The structure of the CPS shown in Fig. 2 was confirmed by analysis of glycoside β-8ePse5Ac7Ac-(2→1)-Gro and a linear GlcNAc polymer (main-chain polysaccharide) obtained by Smith degradation of the CPS (for the NMR chemical shifts of these products see Tables 1 and 2).

The chemical shift for C6 of the higher sugar (δ 72.5) is similar to the C6 chemical shift (73.0 ppm) of β-8ePse5Ac7Ac having the axial carboxyl group but significantly different from that (70.3 ppm) of α-8ePse5Ac7Ac with the equatorial carboxyl group [25]. Therefore, 8-epipseudaminic acid in the CPS has the axial carboxyl group and thus is β-linked.

In the CPS, the higher sugar forms part of an 8ePse5Ac7Ac-(2→6)-Gal disaccharide side chain that branches from a GlcNAc homopolymer main chain. The attachment of the side chain to position 6 of one of the main-chain components, namely α-GlcNAc, was confirmed by a glycosylation effect, that is a low-field position at δ_C 66.5 of the C6 signal of the α-GlcNAc residue in the NMR spectrum of the CPS, as compared with its position at 61.8 ppm in the spectrum of the corresponding non-substituted monosaccharide (Table 1).

2.3. Assignment of glycosyltransferases to linkages reveals the first sugar of the K-unit

In *A. baumannii*, the first sugar of K-unit structures is usually identified via detection of the type of initiating transferase (Itr) encoded by the CPS biosynthesis gene cluster that is responsible for linking the first sugar to the inner membrane lipid carrier, undecaprenol

phosphate (Und-P), thus beginning CPS biosynthesis. The whole genome sequence of RES-546 was obtained by Illumina sequencing and found to include the KL135 gene cluster at the CPS biosynthesis 'K locus' (Supplementary Fig. S1). KL135 encodes ItrA3, which is a known D-Glc*p*NAc-1-P transferase initiating transferase [15]. However, as two D-Glc*p*NAc sugars are present in the K135 main chain (Fig. 2), the first sugar was determined via assignment of the encoded glycosyltransferases that formed the other linkages in the K135 unit.

The linkages formed by two glycosyltransferases, encoded by *gtr14* and *gtr15*, have been predicted previously for the *A. baumannii* K5, K7, K46 and K90 CPS [13, 19]. Gtr14 is predicted to form an α-D-Gal*p*-(1→6)-D-Glc*p*NAc linkage, and the same linkage is present in K135 (Fig. 2). Gtr15 is suggested to have relaxed specificity for its acceptor sugar, having been reported to transfer D-Glc*p*NAc to either a D-Glc*p*NAc or D-Gal*p*NAc acceptor via an α-(1→3) linkage [13]. The type of acceptor is determined by the presence of either ItrA2 or ItrA3, and as an *itrA3* gene is present together with *gtr15* in KL135, Gtr15 would form the α-D-Glc*p*NAc-(1→3)-D-Glc*p*NAc linkage. The remaining glycosyltransferase, Gtr197, would therefore form the β-8ePse5Ac7Ac-(2→6)-D-Gal*p* linkage. Hence, the β-substituted D-Glc*p*NAc in the main chain is the first sugar, identifying β-D-Glc*p*NAc-(1→3)-D-Glc*p*NAc as the linkage between K units that is formed by the Wzy polymerase (Fig. 2).

3. DISCUSSION

The finding of a KL135 CPS biosynthesis gene cluster in the whole genome sequence of *A. baumannii* RES-546 enabled the CPS structure to be named K135. The composition of the K135 CPS was determined by chemical methods and NMR spectroscopy, and the complete CPS structure was established using the latter approach. This finding enabled assignment of glycosyltransferases to the linkages.

As previously reported [20], the K135 CPS contains a D-*glycero*-L-*manno* isomer of 5,7-di-*N*-acetyl-8-epipseudaminic acid that had not been hitherto found in nature. This sugar forms part of a disaccharide that branches from a GlcNAc homopolymer main chain (Fig. 2).

The KL135 gene cluster includes a module of six genes (*psaABCDEF*) for the synthesis of Pse5Ac7Ac [23], the C8 epimer of 8ePse5Ac7Ac. For all *A. baumannii* strains studied to date that include these genes in their genome, Pse5Ac7Ac has been found in the CPS structure [13, 14, 19, 26-29]. However, analysis of the KL135 gene cluster (Supplementary Fig. S1) could not identify additional genes that are necessary for the conversion of Pse5Ac7Ac to 8ePse5Ac7Ac. Hence, further work will be needed to determine the genes required for synthesis of the latter monosaccharide.

4. MATERIALS AND METHODS

4.1. Cultivation of bacteria and isolation of capsular polysaccharide

A. baumannii isolate RES-546 was recovered in 2002 from an intraabdominal patient sample in Irkutsk, Russia [20]. Bacterial cells were cultivated in 2×TY media overnight, then harvested by centrifugation (10,000×*g*, 15 min) and suspended in phosphate buffered saline. The suspension was cooled down to 4 °C, and 0.2 volume of aq 50% CCl_3CO_2H was added. Cells were precipitated by centrifugation (15,000×*g*, 20 min), and two volumes of acetone were added to the supernatant. After intense shaking, a crude CPS preparation was separated by centrifugation (8,000×*g*, 20 min), dissolved in water, and the pH value adjusted to pH 8 by adding 1 M NaOH. CPS was then precipitated with acetone and separated by centrifugation as above. It was then dissolved in distilled water and applied to a column (53 × 3.5 cm) of Sephadex G-50 Superfine (Healthcare). Elution was performed with 0.1% HOAc and monitored using a UV-detector (Uvicord, Sweden) at 206 nm to give purified CPS samples.

4.2. Monosaccharide analysis

CPS samples (1 mg) were hydrolyzed with 2 M CF_3CO_2H (120 °C, 2 h). Monosaccharides were converted conventionally into the alditol acetates analyzed by GLC on a Maestro (Agilent 7820) chromatograph (Interlab, Russia) equipped with an HP-5 column (0.32 mm × 30 m) using a temperature program of 160 °C (1 min) to 290 °C at 7 °C min^{-1}.

4.3. Smith degradation

A CPS sample (54 mg) from *A. baumannii* RES-546 was oxidized with aqueous 0.05 M $NaIO_4$ (1 mL) at 20 °C for 48 h in the dark, reduced with an excess of $NaBH_4$ at 20 °C for 16 h. The excess of $NaBH_4$ was destroyed with concentrated AcOH, the solution was evaporated, and the residue was evaporated with methanol (3 × 1 mL), dissolved in water (in 0.5 mL) and applied to a column (35 × 2 cm) of TSK HW-40. The degraded polysaccharide was eluted with aqueous 0.1% AcOH and hydrolyzed with 2 % HOAc (100 °C, 2 h) to give the β-8ePseAc$_2$-(2→1)-Gro glycoside (5.2 mg) and a linear GlcNAc polymer (main-chain polysaccharide, 12 mg), which were isolated by gel-permeation chromatography on a column (108 × 1.2 cm) of TSK HW-40 in 1% HOAc.

4.4. NMR spectroscopy

Samples were deuterium-exchanged by freeze-drying from 99.9 % D_2O and then examined as solutions in 99.95 % D_2O. NMR spectra were recorded on a Bruker Avance II 600 MHz spectrometer (Germany) at 60 °C. Sodium 3-trimethylsilylpropanoate-2,2,3,3-d_4 (δ_H 0, δ_C −1.6) was used as internal reference for calibration. 2D NMR spectra were obtained using standard Bruker software, and Bruker TopSpin 2.1 program was used to acquire and process the NMR data. 60-ms MLEV-17 spin-lock time and 150-ms mixing time were used in ^{1}H,^{1}H TOCSY and ROESY experiments, respectively. A 60-ms delay was used for evolution of

long-range couplings to optimize $^{1}H,^{13}C$ HMBC experiments for the coupling constant of $J_{H,C}$ 8 Hz. ^{1}H and ^{13}C chemical shifts were assigned using two-dimensional $^{1}H,^{1}H$ COSY, $^{1}H,^{1}H$ TOCSY, and $^{1}H,^{13}C$ HSQC experiments (Table 1 and Table 2, Supplementary Fig. S2).

4.5. Assignment of glycosyltransferases

Genomic material from RES-546 was extracted and sequenced on a MiSeq platform using a Nextera DNA library preparation kit (Illumina, San Diego, CA). Short read data was assembled into contigs using SPAdes *v 3.10* [30], and coding sequences were translated and annotated using Prokka *v 1.14.15* [31]. The final annotated assembly of RES-546 was deposited to NCBI under accession number JAMGSJ000000000.1. The CPS biosynthesis gene cluster located at the chromosomal K locus between *fkpA* and *lldP* genes was identified using command-line *Kaptive v 2.1.0* with the most recent iteration of the *A. baumannii* KL reference database that includes 241 KL types in [23]. Roles of encoded glycosyltransferases were predicted by homology to known Gtrs from *A. baumannii* or other species using BLASTp (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

Acknowledgements

NMR spectra were recorded in the Department of Structural Studies of N.D. Zelinsky Institute of Organic Chemistry, Moscow.

Funding

This work was supported by the Russian Science Foundation (grant number 19-14-00273) and an Australian Research Council (ARC) DECRA Fellowship DE180101563.

References

[1] C. J. L. Murray, K. S. Ikuta, F. Sharara, L. Swetschinski, G. R. Aguilar, A. Gray, et al. Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. The Lancet 399 (2022) 629-655.

[2] E. Tacconelli, E. Carrara, A. Savoldi, S. Harbarth, M. Mendelson, D. L. Monnet, et al., Discovery, research, and development of new antibiotics: the WHO priority list of antibiotic-resistant bacteria and tuberculosis. The Lancet Infectious Diseases 18 (2018) 318-327.

[3] T. Russo, J. Beanan, R. Olson, U. MacDonald, A. Cox, F. St. Michael, et al. The K1 capsular polysaccharide from *Acinetobacter baumannii* is a potential therapeutic target via passive immunization. Infect. Immun. 81 (2013) 915-922.

[4] S. X. Wang-Lin, R. Olson, J. M. Beanan, U. MacDonald, T. A. Russo, and J. P. Balthasar. Antibody dependent enhancement of *Acinetobacter baumannii* infection in a mouse pneumonia model. J. Pharmacol. Exp. Ther. 368 (2019) 475-489.

[5] R. Schooley, B. Biswas, J. Gill, A. Hernandez-Morales, J. Lancaster, L. Lessor, et al. Development and use of personalized bacteriophage-based therapeutic cocktails to treat a patient with a disseminated resistant *Acinetobacter baumannii* infection. Antimicrob. Ag. Chemother. 61 (2017) e00954-17.

[6] H. Oliveira, A. Mendes, A. G. Fraga, A. Ferreira, A. I. Pimenta, D. Mil-Homens, et al. K2 capsule depolymerase is highly stable, is refractory to resistance, and protects larvae and mice from *Acinetobacter baumannii* sepsis. Appl. Environ. Microbiol. 85 (2019) e00934-19.

[7] A. V. Popova, M. M. Shneider, N. P. Arbatsky, A. A. Kasimova, S. N. Senchenkova, A. S. Shashkov, et al. Specific interaction of novel Friunavirus phages encoding

tailspike depolymerases with corresponding *Acinetobacter baumannii* capsular types. J. Virol. 95 (2021) e01714-20.

[8] T. Russo, N. Luke, J. Beanan, R. Olson, S. Sauberan, U. MacDonald, et al. The K1 capsular polysaccharide of *Acinetobacter baumannii* strain 307-0294 is a major virulence factor. Infect. Immun. 78 (2010) 3993-4000.

[9] Y. Talyansky, T. B. Nielsen, J. Yan, U. Carlino-Macdonald, G. Di Venanzio, S. Chakravorty, et al. Capsule carbohydrate structure determines virulence in *Acinetobacter baumannii.* PLoS Pathog. 17 (2021) e1009291.

[10] A. A. Kasimova, N. P. Arbatsky, O. Y. Timoshina, M. M. Shneider, A. S. Shashkov, A. O. Chizhov, et al. The K26 capsular polysaccharide from *Acinetobacter baumannii* KZ-1098: Structure and cleavage by a specific phage depolymerase. Int. J. Biol. Macromol. 191 (2021) 182-191.

[11] J. J. Kenyon, A. A. Kasimova, A. S. Shashkov, R. M. Hall, and Y. A. Knirel. *Acinetobacter baumannii* isolate BAL_212 from Vietnam produces the K57 capsular polysaccharide containing a rarely occurring amino sugar N-acetylviosamine. Microbiol. 164 (2018) 217-220.

[12] N. P. Arbatsky, J. J. Kenyon, A. A. Kasimova, A. S. Shashkov, M. M. Shneider, A. V. Popova, et al. K units of the K8 and K54 capsular polysaccharides produced by *Acinetobacter baumannii* BAL 097 and RCH52 have the same structure but contain different di-N-acyl derivatives of legionaminic acid and are linked differently. Carbohydr. Res. 483 (2019) 107745.

[13] S. N. Senchenkova, J. J. Kenyon, T. Jia, A. V. Popova, M. M. Shneider, A. A. Kasimova, et al. The K90 capsular polysaccharide produced by *Acinetobacter baumannii* LUH5553 contains di-N-acetylpseudaminic acid and is structurally related

to the K7 polysaccharide from *A. baumannii* LUH5533. Carbohydr. Res. 479 (2019) 1-5.

[14] J. J. Kenyon, A. M. Marzaioli, R. M. Hall, and C. De Castro. Structure of the K2 capsule associated with the KL2 gene cluster of *Acinetobacter baumannii*. Glycobiology. 24 (2014) 554-563.

[15] J. J. Kenyon, A. S. Shashkov, S. N. Senchenkova, M. M. Shneider, B. Liu, A. V. Popova, et al. *Acinetobacter baumannii* K11 and K83 capsular polysaccharides have the same 6-deoxy-L-talose-containing pentasaccharide K units but different linkages between the K units. Int. J. Biol. Macromol. 103 (2017) 648-655.

[16] A. A. Kasimova, J. J. Kenyon, N. P. Arbatsky, A. S. Shashkov, A. V. Popova, M. M. Shneider, et al. *Acinetobacter baumannii* K20 and K21 capsular polysaccharide structures establish roles for UDP-glucose dehydrogenase Ugd2, pyruvyl transferase Ptr2 and two glycosyltransferases. Glycobiology. 28 (2018) 876-884.

[17] N. P. Arbatsky, A. A. Kasimova, A. S. Shashkov, M. M. Shneider, A. V. Popova, D. A. Shagin, et al., Involvement of a phage-encoded Wzy protein in the polymerization of K127 units to form the capsular polysaccharide of *Acinetobacter baumannii* isolate 36-1454. Microbiol. Spectr. 27 (2022) e0150321.

[18] J. J. Kenyon, A. A. Kasimova, A. Notaro, N. P. Arbatsky, I. Speciale, A. S. Shashkov, et al. *Acinetobacter baumannii* K13 and K73 capsular polysaccharides differ only in K-unit side branches of novel non-2-ulosonic acids: di-N-acetylated forms of either acinetaminic acid or 8-epiacinetaminic acid. Carbohydr. Res. 452 (2017) 149-155.

[19] J. J. Kenyon, N. P. Arbatsky, M. M. Shneider, A. V. Popova, A. S. Dmitrenok, A. A. Kasimova, et al. The K46 and K5 capsular polysaccharides produced by *Acinetobacter baumannii* NIPH 329 and SDF have related structures and the side-

chain non-ulosonic acids are 4-O-acetylated by phage-encoded O-acetyltransferases. PLoS One. 14 (2019) e0218461.

[20] A. S. Shashkov, Arbatsky, N.P., Sof'ya, N.S., Perepelov, A.V., Chizhov, A.O., Dmitrenok, A.S., Shneider, M.M. and Knirel, Y.A. Identification of 5,7-diacetamido-3,5,7,9-tetradeoxy-D-glycero-L-manno-non-2-ulosonic acid (di-*N*-acetyl-8-epipseudaminic acid) in the capsular polysaccharide of *Acinetobacter baumannii* Res546. Carbohydr. Res. 513 (2022) 108531.

[21] J. J. Kenyon and R. M. Hall. Variation in the complex carbohydrate biosynthesis loci of *Acinetobacter baumannii* genomes. PLoS One. 8 (2013) e62160.

[22] K. L. Wyres, S. M. Cahill, K. E. Holt, R. M. Hall, and J. J. Kenyon. Identification of *Acinetobacter baumannii* loci for capsular polysaccharide (KL) and lipooligosaccharide outer core (OCL) synthesis in genome assemblies using curated reference databases compatible with Kaptive. Microb. Genom. 6 (2020) e000339.

[23] S. M. Cahill, R. M. Hall, and J. J. Kenyon. An update to the database for *Acinetobacter baumannii* capsular polysaccharide locus typing extends the extensive and diverse repertoire of genes found at and outside the K locus. bioRxiv 2022.05.19.492579.

[24] G. M. Lipkind, A. S. Shashkov, Y. A. Knirel, E. V. Vinogradov, N. K. Kochetkov. A computer-assisted structural analysis of regular polysaccharides on the basis of ^{13}C-n.m.r. data. *Carbohydr. Res.* V. 175, No. 1, 1988, 59-75].

[25] Y. A. Knirel, A. S. Shashkov, Y. Tsvetkov, P. Jansson, and U. Zähringer. 5,7-diamino-3,5,7,9-tetradeoxynon-2-ulosonic acids in bacterial glycopolymers: chemistry and biochemistry. Adv. Carbohydr. Chem. Biochem. 58 (2003) 371-417.

[26] S. N. Senchenkova, A. S. Shashkov, M. M. Shneider, N. P. Arbatsky, A. V. Popova, K. A. Miroshnikov*, e*t al. Structure of the capsular polysaccharide of *Acinetobacter*

baumannii ACICU containing di-*N*-acetylpseudaminic acid. Carbohydr. Res. 391 (2014) 89-92.

[27] J. J. Kenyon, Marzaioli, A.M., Hall, R.M. and De Castro, C. Structure of the K6 capsular polysaccharide from *Acinetobacter baumannii* isolate RBH4. Carbohydr. Res. 409 (2015) 30-35.

[28] J. J. Kenyon, N. P. Arbatsky, E. L. Sweeney, A. S. Shashkov, M. M. Shneider, A. V. Popova, et al. Production of the K16 capsular polysaccharide by *Acinetobacter baumannii* ST25 isolate D4 involves a novel glycosyltransferase encoded in the KL16 gene cluster. Int. J. Biol. Macromol. 128 (2019) 101-106.

[29] N. P. Arbatsky, M. M. Shneider, A. S. Shashkov, A. V. Popova, K. A. Miroshnikov, N. Volozhantsev, et al. Structure of the *N*-acetylpseudaminic acid-containing capsular polysaccharide of *Acinetobacter baumannii* NIPH67. Russ. Chem. Bull. 65 (2016) 588–591.

[30] A. Bankevich, S. Nurk, D. Antipov, A. Gurevich, M. Dvorkin, A. Kulikov, et al. SPAdes: A new genome assembly algorithm and its applications to single-cell sequencing. J. Comput. Biol. 19 (2012) 455–477.

[31] T. Seemann. Prokka: rapid prokaryotic genome annotation. Bioinformatics. 30 (2014) 2068–2069.

Table 1. ^{13}C NMR chemical shifts (ppm)

Sugar	^{13}C NMR chemical shifts						
Glycoside β-8ePseAc$_2$-(2→1)-Gro **1** from the CPS of *A. baumannii* RES-546							
	C3	C4	C5	C6	C7	C8	C9
β-8ePseAc$_2$ **D**	37.1	67.9	49.2	72.5	54.3	66.9	18.0
	C1	C2	C3				
-1)-Gro	66.6	71.9	63.9				
Main-chain polysaccharide **2** from the CPS of *A. baumannii* RES-546							
	C1	C2	C3	C4	C5	C6	
-3)-α-GlcNAc **B**	98.2	53.6	79.4	72.2	73.3	61.8	
-3)-β-GlcNAc **A**	101.8	55.7	80.6	69.6	76.8	61.6	
CPS of *A. baumannii* RES-546							
	C3	C4	C5	C6	C7	C8	C9
β-8ePseAc$_2$ **D**	37.2	68.0	49.3	72.5	54.3	66.9	18.1
	C1	C2	C3	C4	C5	C6	
-6)-α-Gal **C**	99.6	69.9	70.7	70.7	72.0	65.1	
-3,6)-α-GlcNAc **B**	98.5	53.7	80.1	72.0	70.7	66.5	
-3)-β-GlcNAc **A**	101.8	55.8	80.7	69.2	76.7	61.6	

Table 2. ^{1}H NMR chemical shifts (ppm)

Sugar	^{1}H NMR chemical shifts						
Glycoside β-8ePseAc$_2$-(2→1)-Gro **1** from the CPS of *A. baumannii* RES-546							
	H3ax,H3eq	H4	H5	H6	H7	H8	H9
β-8ePseAc$_2$ **D**	1.64, 2.53	3.88	4.19	3.86	3.91	4.39	1.08
	H1a,H1b	H2	H3a,H3b				
-1)-Gro	3.49, 3.76	3.86	3.57, 3.64				
Main-chain polysaccharide **2** from the CPS of *A. baumannii* RES-546							
	H1	H2	H3	H4	H5	H6a,H6b	
-3)-α-GlcNAc **B**	5.38	3.99	3.80	3.56	3.63	3.37, 3.82	
-3)-β-GlcNAc **A**	4.60	3.79	3.78	3.73	3.48	3.76, 3.92	
CPS of *A. baumannii* RES-546							
	H3ax,H3eq	H4	H5	H6	H7	H8	H9
β-8ePseAc$_2$ **D**	1.59, 2.52	3.88	4.18	3.85	3.92	4.39	1.08
	H1	H2	H3	H4	H5	H6a,H6b	
-6)-α-Gal **C**	5.00	3.84	3.92	3.89	3.96	3.52, 3.98	
-3,6)-α-GlcNAc **B**	5.36	4.02	3.80	3.70	4.01	3.67, 4.08	
-3)-β-GlcNAc **A**	4.62	3.79	3.78	3.73	3.48	3.76, 3.92	

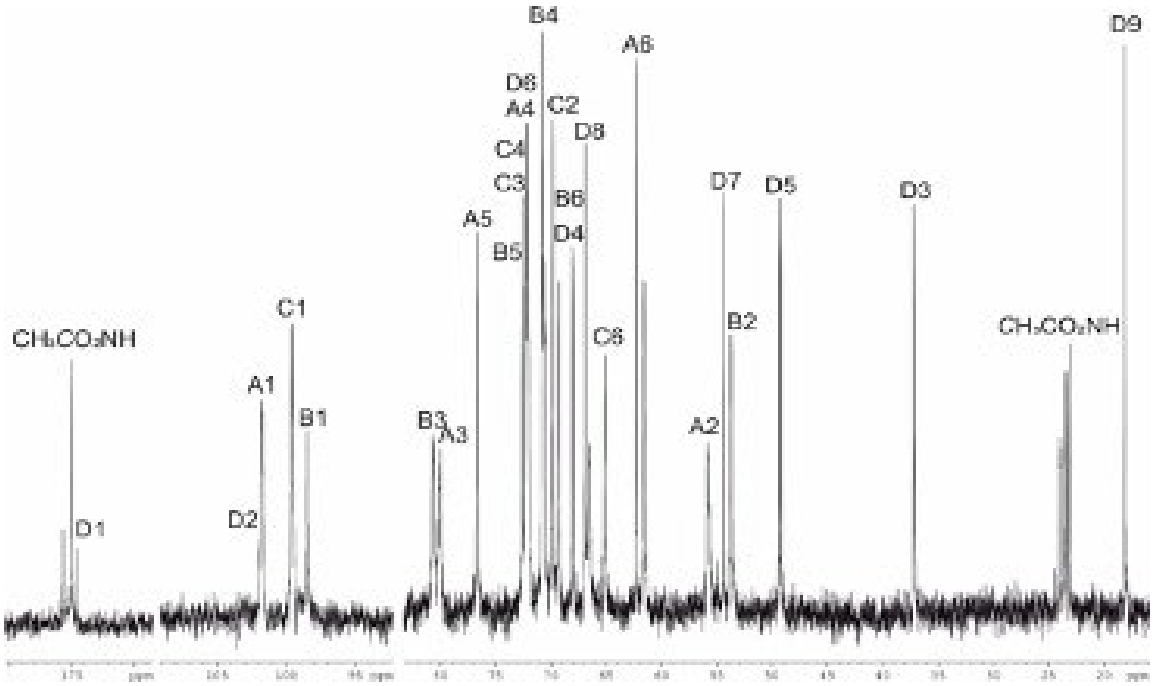

Figure 1. ^{13}C NMR spectrum of the CPS of *A. baumannii* RES-546. For designation of the sugar residues see Table 1.

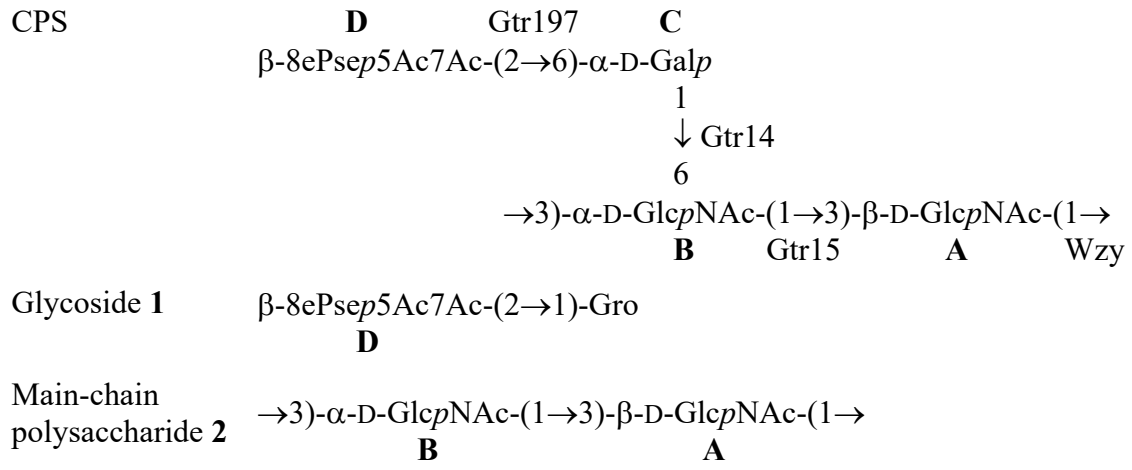

Figure 2. Structures of the K135 CPS from *A. baumannii* RES-546 and products of Smith degradation of the CPS (glycoside **1** and main-chain polysaccharide **2**). 8ePse5Ac7Ac indicates 5,7-diacetamido-3,5,7,9-tetradeoxy-D-*glycero*-L-*manno*-non-2-ulosonic acid (di-*N*-acetyl-8-epipseudaminic acid).

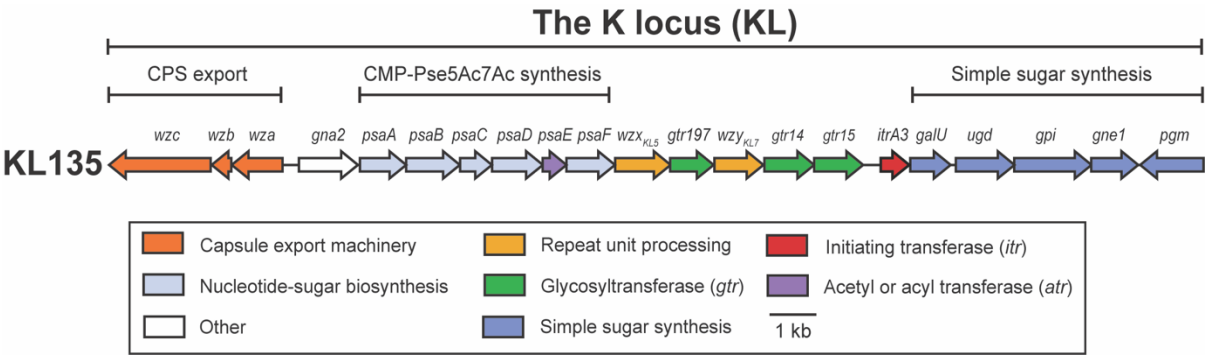

Figure S1. Arrangement of the KL135 CPS biosynthesis gene cluster found at the K locus in the RES-546 chromosome. Figure is drawn to scale from GenBank accession number OM782677.1. Colour scheme for genes (shown below) denotes functions of gene products. Scale is shown below.

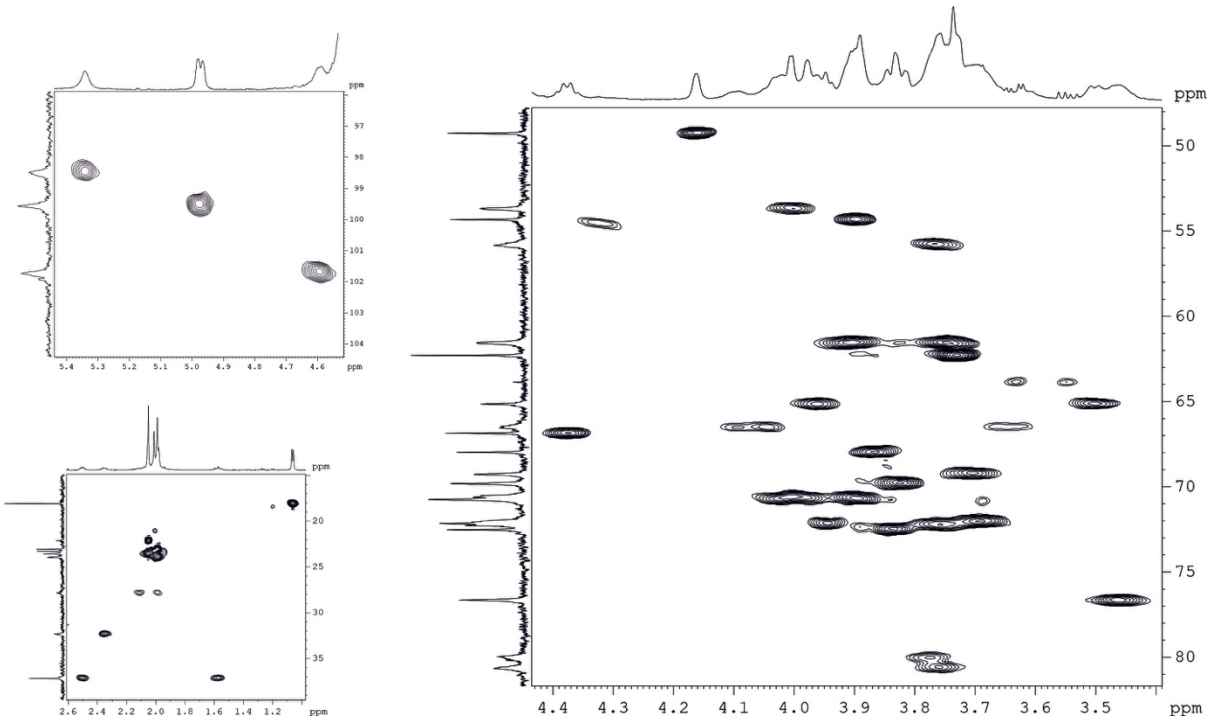

Figure S2. Parts of a two-dimensional $^{1}H,^{13}C$ HSQC spectrum of the CPS of *A. baumannii* RES-546. The corresponding parts of the ^{1}H and ^{13}C NMR spectra are shown along the horizontal and vertical axes, respectively.