# Structure of the capsular polysaccharide of *Acinetobacter baumannii* MAR 55-66

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A capsular polysaccharide was isolated from cells of opportunistic bacterial pathogen *Acinetobacter baumannii* strain MAR 55-66. According to carbohydrate analysis and $^{1}H$ and $^{13}C$ NMR spectroscopy, it is composed of heptasaccharide repeating units, which include five L-rhamnose (Rha) residues and one residue each of D-glucuronic acid (GlcA) and *N*-acetyl-D-glucosamine (GlcNAc). For structural analysis of this polysaccharide, the Smith degradation, depolymerization with a recombinant *endo*-glycosidase from a specific bacteriophage, and selective solvolysis with $CF_3CO_2H$ were applied. The two last methods resulted in formation of the same linear heptasaccharide, which was subjected to the following chemical modifications: borohydride reduction, β-elimination with alkali, and partial acid hydrolysis. The resulting oligosaccharides were isolated by gel-permeation chromatography, and their structures were established by $^{1}H$ and $^{13}C$ NMR spectroscopy including 2D $^{1}H—^{1}H$ and $^{1}H—^{13}C$ NMR correlation experiments, as well as high-resolution electrospray ionization mass spectrometry. Based on the data obtained, the structure of the branched heptasaccharide repeating unit containing three Rha residues and one GlcNAc residue in the main chain along with two Rha residues and one GlcA residue in the side chain was established. The polysaccharide studied belongs to a group of structurally similar capsular polysaccharides of *A. baumannii,* which are built up of branched oligosaccharide repeating units that include four or five L-Rha residues and one residue each of D-GlcA and D-GlcNAc.

**Key words:** *Acinetobacter baumannii*, capsular polysaccharide, structure, depolymerization, oligosaccharide.

An opportunistic pathogen species of *Acinetobacter baumannii* belongs to a group of nosocomial pathogens that are highly resistant to antibiotics and cause most of hospital infections. One of the factors of virulence of this pathogen is a capsular polysaccharide (CPS). It forms a surface layer, which protects the bacterial cell from host immune response, dehydration, and assists in survival under unfavorable conditions. Fine structure of CPS determines the specificity of the immune response against infection and interactions of bacteria with bacteriophages. The capsular polysaccharide is interesting as a target for new agents of immunotherapy and phage therapy of infectious diseases caused by *A. baumannii*.

Capsular polysaccharides of *A. baumannii* are highly variable owing to a variability of the genetic K locus responsible for biosynthesis of the capsule. Genetic data provide the evidence of existence of at least of 130 capsular types of bacteria, from which about 40 CPS structures have been characterized (see Database of Bacterial Carbohydrates, http://csdb.glycoscience.ru/bacterial). This work is a continuation of our studies of the CPS structures of *A. baumannii*[1,2] (see also literature cited in these papers) and is devoted to determination of the CPS structure of strain MAR 55-66 isolated in 2015.

## Results and Discussion

The capsular polysaccharide was isolated from cells of *A. baumannii* MAR 55-66 by phenol—water extraction[3] followed by precipitation of accompanying proteins and nucleic acids with $CCl_3CO_2H$ and gel-permeation chromatography on Sephadex G-50.[4] Monosaccharide analysis showed that the CPS contained rhamnose (Rha), glucuronic acid (GlcA), and glucosamine (GlcN). The L configuration of rhamnose and the D configuration of GlcA and GlcN, which are common for these monosaccharides in the CPSs of *A. baumannii*, were inferred from analysis of the biosynthesis gene clusters of the CPSs of this strain (these data will be published elsewhere).

In the $^{1}H$ NMR spectrum of the polysaccharide, there were present signals of seven anomeric protons at $\delta_H$ 4.73—5.17, five methyl groups of the rhamnose residues at $\delta_H$

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1.24—1.31, and one *N*-acetyl group at $\delta_H$ 2.03. In the $^{13}C$ NMR spectrum, signals of seven anomeric carbon atoms at $\delta_C$ 102.1—104.9, five methyl groups of the rhamnose residues at $\delta_C$ 17.7—18.1, one *N*-acetyl group signal at $\delta_C$ 23.5 (Me) and 175.5 (CO), one $CH_2OH$ group of GlcNAc at $\delta_C$ 62.1, and one $CO_2H$ group of GlcA at $\delta_C$ 174.0 were present. Therefore, the repeating unit of the CPS consists of seven monosaccharide residues including five Rha residues, one GlcNAc residue, and one GlcA residue.

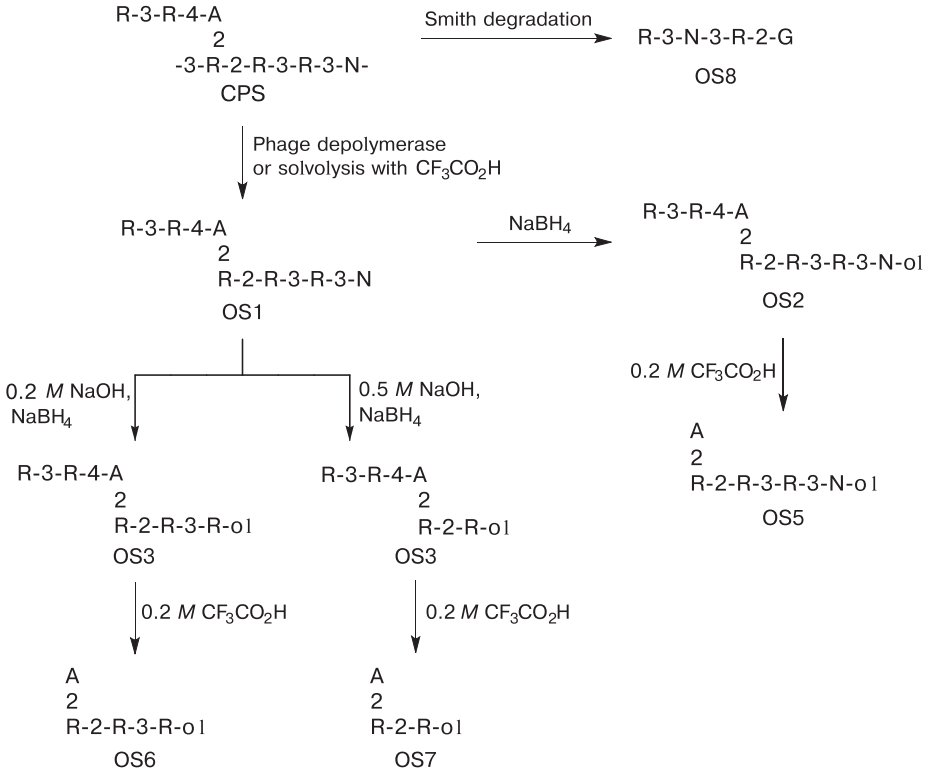
The most effective and modern approach to structural analysis of complex polysaccharides is a physicochemical method based on total assignment of signals in the $^1H$ and $^{13}C$ NMR spectra using 2D NMR experiments.[5] However, application of this approach to structural analysis of the CPS studied was impeded by the presence of a large number of Rha residues giving NMR signals with similar or identical chemical shifts. Therefore, selective cleavages of the CPS followed by chemical modifications of the resulting linear heptasaccharide, which corresponded to the repeating unit of the CPS, and the Smith degradation were carried out.

For the cleavage of the CPS, a recombinant protein from tail spike of vB_AbaP_APK86 bacteriophage with polysaccharide-depolymerase activity was used. As a result, the following reducing oligosaccharides were obtained as main products: heptasaccharide OS1 corresponding to the repeating unit of the CPS and tetradecasaccharide, which is its dimer. Therefore, depolymerase of vB_AbaP_APK86 bacteriophage is a glycosidase that cleaves the CPS by hydrolytic mechanism. Oligosaccharide OS1 was also formed by solvolysis of the CPS with anhydrous $CF_3CO_2H$.[6] Borohydride reduction of OS1 afforded oligosaccharide OS2 (Scheme 1).

Heptasaccharide OS1 possessing at the reducing end the →3)-Rha-(1→3)-GlcNAc disaccharide fragment with 3-*O*-substituted monosaccharide residues was subjected to β-elimination under different alkaline conditions (see Scheme 1). Under relatively mild conditions (0.1 *M* NaOH, 40 °C, 1 h), removal of only GlcNAc residue occurred and after reduction with $NaBH_4$ oligosaccharide OS3 was obtained. Under more harsh conditions (0.5 *M* NaOH, 50 °C, 2 h), GlcNAc and Rha residues were cleaved and subsequent borohydride reduction afforded OS4 as the main product.

Mild acid hydrolysis of OS2, OS3, and OS4 with 0.2 *M* $CF_3CO_2H$ (100 °C, 1 h) resulted in selective cleavage of the terminal disaccharide, which consisted of two Rha

**Scheme 1**




*Notes.* Selective degradations of the CPS from *A. baumannii* strain MAR 55-66 and chemical modifications of the derived oligosaccharides OS1—OS8. Structures of the CPS and OS1—OS8 are given in Fig. 1. A is glucuronic acid; G is glyceraldehyde hydrate; N is 2-acetamido-2-deoxyglucose; N-ol is 2-acetamido-2-deoxyglucitol; R is rhamnose; R-ol is rhamnitol.

residues, to give oligosaccharides OS5, OS6, and OS7, respectively (see Scheme 1).

To reveal the type of the linkage between the heptasaccharide repeating units, it was necessary to obtain an oligosaccharide containing the first monosaccharide of the repeating unit (GlcNAc) with the intact glycosidic bond connecting it to the adjacent repeating unit. For this purpose, the Smith degradation of CPS was done. The procedure included periodate oxidation of 2-substituted Rha and 4-substituted GlcA residues followed by borohydride reduction of the resulting polyaldehyde and mild acid hydrolysis of the degraded monosaccharide residues. As a result, a glycoside of the trisaccharide (OS8) containing two Rha and one GlcNAc residues along with glyceraldehyde hydrate (Gro-al) as an aglycone was obtained (see Scheme 1).

The structures of the oligosaccharides (Fig. 1) were established by 2D homonuclear and heteronuclear NMR spectroscopy.[5] The assignment of $^1$H and $^{13}$C NMR signals was carried out using $^1$H—$^1$H COSY, $^1$H—$^1$H TOCSY, and $^1$H—$^{13}$C HSQC experiments (Table 1). The glycosylation points and monosaccharide sequence were determined by correlation of the signals of the adjacent monosaccharide residues revealed by $^1$H—$^1$H ROESY and $^1$H—$^{13}$C HMBC spectra (Table 2). The structures of the oligosaccharides presented in Fig. 1 were supported by determination of their molecular masses by high-resolution electrospray ionization mass spectrometry[8] (Table 3).

Elucidation of the structure of OS8 showed that both action of the phage depolymerase and solvolysis of the CPS with anhydrous $CF_3CO_2H$ resulted in selective cleavage of the glycosidic bond between β-GlcNAc and 2,3-disubstituted Rha residues. This selectivity showed that the bond of GlcNAc is cleaved easier than those of Rha*p*. This fact is apparently accounted for by a peculiarity of the CPS structure, namely, by the β-configuration of the glycosidic bond of the GlcNAc residue, which is less stable than that of the α-configuration, and the attachment of this monosaccharide to a vicinal disubstituted Rha residue.

Using the NMR data of the oligosaccharides enabled assignment of the $^1$H and $^{13}$C NMR signals in the spectra of the initial CPS (see Table 1) and identification of the inter-residue correlation peaks in its 2D $^1$H—$^1$H ROESY and $^1$H—$^{13}$H HMBC NMR spectra (see Table 2). As a result, the structure of the CPS of *A. baumannii* MAR 55-66 CPS was established (see Fig. 1), which was in a good accordance with the structures of the oligosaccharides OS1—OS8 obtained from this CPS.

The CPS studied is structurally related to the previously explored group of the CPSs of *A. baumannii*, which have the same monosaccharide composition and branched heptasaccharide or octasaccharide repeating units with

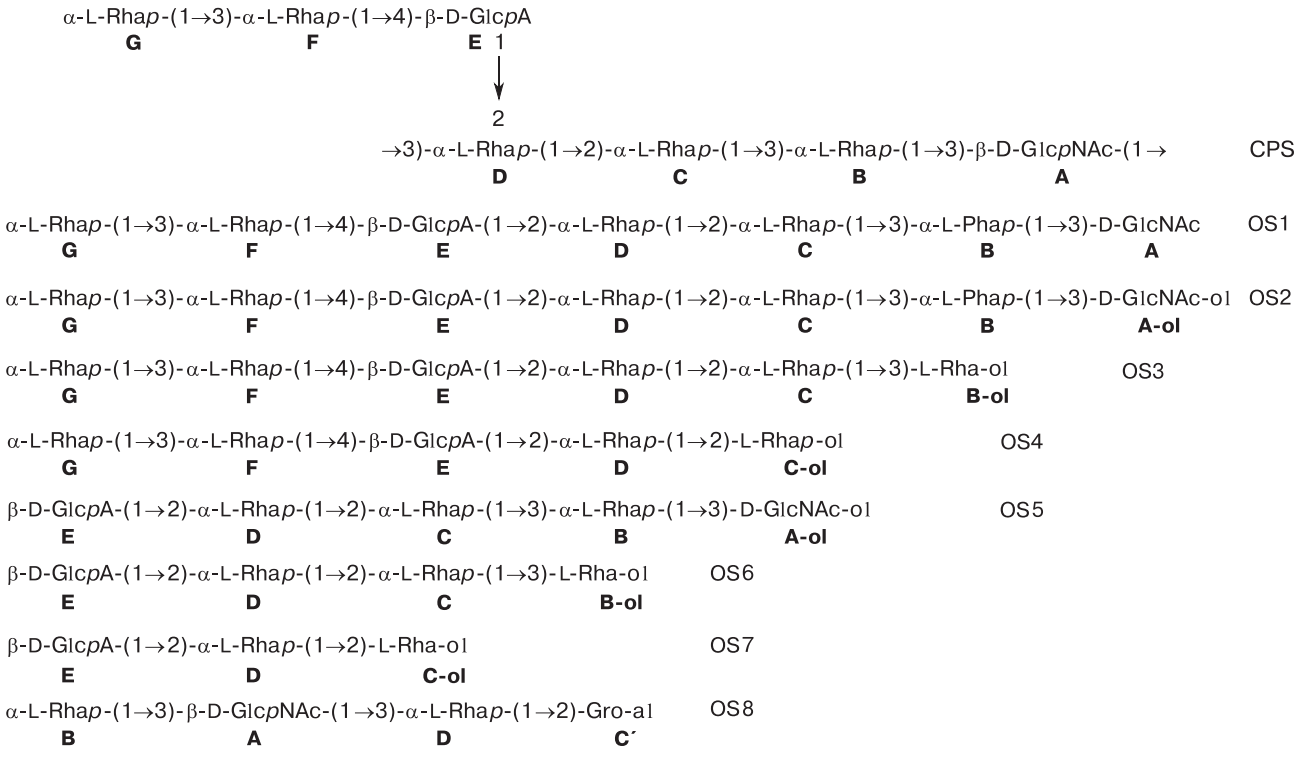



**Fig. 1**. Structures of the CPS from *A.baumanii* strain MAR 55-66 and oligosaccharides OS1—OS8 obtained from the CPS. GlcA is glucuronic acid; GlcNAc is 2-acetamido-2-deoxyglucose (*N*-acetylglucosamine); GlcNAc-ol is 2-acetamido-2-deoxyglucitol; Gro-al is glyceraldehyde hydrate; Rha is rhamnose; Rha-ol is rhamnitol.

**Table 1**. $^{1}H$ and $^{13}C$ NMR chemical shifts (δ) of the CPS from *A. baumannii* strain MAR 55-66 and related oligosaccharides OS1-OS8 (see Fig. 1)

| Monosaccharide residue | C(1) H(1) (1a,1b) | C(2) H(2) | C(3) H(3) (3a,3b) | C(4) H(4) | C(5) H(5) | C(6) H(6) (6a,6b) |
|---|---|---|---|---|---|---|
| | | | CPS | | | |
| **G** | *103.6* | *71.5* | *71.6* | *73.5* | *70.2* | — |
| | 5.02 | 4.06 | 3.83 | 3.46 | 3.79 | — |
| **F** | *102.2* | *71.5* | *79.3* | *72.5* | *70.6* | — |
| | 4.74 | 3.98 | 3.78 | 3.52 | 4.04 | — |
| **E** | *104.9* | *74.8* | *75.4* | *80.5* | *75.4* | *174.0* |
| | 4.79 | 3.38 | 3.63 | 3.66 | 3.98 | — |
| **D** | *102.4* | *79.5* | *81.2* | *72.1* | *70.3* | — |
| | 5.17 | 4.40 | 3.91 | 3.56 | 3.72 | — |
| **C** | *102.1* | *79.3* | *71.3* | *73.4* | *70.3* | — |
| | 5.14 | 4.04 | 3.90 | 3.54 | 3.76 | — |
| **B** | *102.5* | *71.9* | *78.7* | *72.9* | *70.5* | — |
| | 4.86 | 3.85 | 3.78 | 3.56 | 4.01 | — |
| **A** | *103.4* | *56.8* | *82.8* | *69.6* | *77.1* | *62.1* |
| | 4.73 | 3.87 | 3.64 | 3.57 | 3.49 | 3.78, 3.95 |
| | | | OS1 | | | |
| **G** | *103.5* | *71.5* | *72.0* | *73.5* | *70.3* | — |
| | 5.04 | 4.07 | 3.84 | 3.47 | 3.76 | — |
| **F** | *101.7* | *71.5* | *79.4* | *72.6* | *70.3* | — |
| | 4.75 | 4.02 | 3.80 | 3.53 | 4.06 | — |
| **E** | *105.5* | *75.0* | *75.4* | *80.2* | *77.7* | *176.4* |
| | 4.61 | 3.43 | 3.59 | 3.61 | 3.74 | — |
| **D** | *102.3* | *81.3* | *71.4* | *73.4* | *70.3* | — |
| | 5.25 | 4.16 | 3.88 | 3.48 | 3.74 | — |
| **C** | *102.0* | *79.6* | *71.4* | *73.7* | *70.3* | — |
| | 5.16 | 4.05 | 3.92 | 3.50 | 3.83 | — |
| **B** | *102.3** | *72.0* | *78.7* | *72.6* | *71.2* | — |
| | 4.87 * | 3.83 | 3.77 | 3.56 | 3.87 | — |
| **A**a | *92.4* | *54.8* | *80.6* | *69.9* | *72.9* | *62.*1 |
| | 5.15 | 4.04 | 3.79 | 3.57 | 3.88 | 3.79, 3.86 |
| **A**b | *95.8* | *57.7* | *83.0* | *69.8* | *77.4* | *62.*2 |
| | 4.74 | 3.79 | 3.61 | 3.56 | 3.48 | 3.77, 3.92 |
| | | | OS2 | | | |
| **G** | *103.6* | *71.6* | *71.7* | *73.5* | *70.5* | — |
| | 5.04 | 4.07 | 3.84 | 3.46 | 3.78 | — |
| **F** | *101.8* | *71.5* | *79.5* | *72.7* | *70.4* | — |
| | 4.75 | 4.02 | 3.80 | 3.53 | 4.07 | — |
| **E** | *105.6* | *75.1* | *75.5* | *80.3* | *77.8* | *176.5* |
| | 4.61 | 3.43 | 3.59 | 3.62 | 3.74 | — |
| **D** | *102.4* | *81.4* | *71.2* | *73.8* | *70.4* | — |
| | 5.26 | 4.16 | 3.88 | 3.47 | 3.73 | — |
| **C** | *102.1* | *79.8* | *71.2* | *73.8* | *70.4* | — |
| | 5.20 | 4.06 | 3.93 | 3.50 | 3.83 | — |
| **B** | *102.7* | *72.1* | *78.8* | *72.8* | *71.2* | — |
| | 4.96 | 3.97 | 3.84 | 3.59 | 3.86 | — |
| **A-ol** | *61.9* | *54.4* | *78.6* | *72.4* | *71.7* | *64.3* |
| | 3.70, 3.74 | 4.24 | 4.08 | 3.69 | 3.64 | 3.69, 3.85 |
| | | | OS3 | | | |
| **G** | *103.5* | *71.5* | *71.5* | *73.4* | *70.3* | — |
| | 5.04 | 4.07 | 3.85 | 3.48 | 3.83 | — |
| **F** | *101.7* | *71.4* | *79.4* | *72.6* | *70.2* | — |
| | 4.75 | 4.03 | 3.81 | 3.53 | 4.08 | — |
| **E** | *105.4* | *74.9* | *75.4* | *80.2* | *77.7* | *176.4* |
| | 4.61 | 3.443 | 3.60 | 3.62 | 3.76 | — |
| **D** | *102.2* | *81.3* | *70.6* | *73.3* | *70.2* | — |
| | 5.26 | 4.16 | 3.87 | 3.49 | 3.73 | — |

(*to be continued*)

**Table 1** (*continued*)

| Monosaccharide residue | C(1) H(1) (1a,1b) | C(2) H(2) | C(3) H(3) (3a,3b) | C(4) H(4) | C(5) H(5) | C(6) H(6) (6a,6b) |
|---|---|---|---|---|---|---|
| **C** | *101.9* | *79.8* | *71.2* | *73.7* | *71.0* | — |
| | 5.08 | 4.04 | 3.89 | 3.51 | 3.87 | — |
| **B-ol** | *64.0* | *73.8* | *79.8* | *75.9* | *68.1* | *20.4* |
| | 3.65, 3.83 | 3.90 | 3.93 | 3.61 | 3.74 | 1.29 |
| | | | OS4 | | | |
| **G** | *103.7* | *71.6* | *71.7* | *73.5* | *70.4* | — |
| | 5.02 | 4.06 | 3.82 | 3.48 | 3.79 | — |
| **F** | *102.3* | *71.6* | *79.3* | *72.6* | *70.7* | — |
| | 4.74 | 3.99 | 3.79 | 3.49 | 4.03 | — |
| **E** | *105.5* | *74.9* | *75.5* | *80.2* | *75.6* | *174.3* |
| | 4.67 | 3.44 | 3.63 | 3.67 | 4.02 | — |
| **D** | *101.0* | *81.4* | *71.5* | *73.5* | *70.4* | — |
| | 5.15 | 4.06 | 3.88 | 3.46 | 3.83 | — |
| **C-ol** | *62.5* | *80.2* | *69.5* | *73.7* | *68.9* | *20.0* |
| | 3.83 | 3.73 | 3.94 | 3.50 | 3.85 | 1.24 |
| | | | OS5 | | | |
| **E** | *105.4* | *74.6* | *73.0* | *76.7* | *77.5* | *176.5* |
| | 4.62 | 3.40 | 3.52 | 3.53 | 3.72 | — |
| **D** | *102.1* | *81.2* | *71.3* | *73.4* | *70.1* | — |
| | 5.29 | 4.18 | 3.90 | 3.51 | 3.74 | — |
| **C** | *102.0* | *79.7* | *71.0* | *73.6* | *70.5* | — |
| | 5.21 | 4.10 | 3.95 | 3.50 | 3.86 | — |
| **B** | *103.4* | *71.5* | *79.4* | *72.4* | *71.9* | — |
| | 4.95 | 4.12 | 3.87 | 3.60 | 3.78 | — |
| **A-ol** | *61.1* | *55.9* | *77.2* | *73.4* | *72.4* | *64.0* |
| | 3.82, 3.90 | 3.68 | 4.29 | 3.75 | 3.69 | 3.71, 3.86 |
| | | | OS6 | | | |
| **E** | *105.5* | *74.7* | *76.9* | *73.1* | *77.6* | *176.3* |
| | 4.63 | 3.41 | 3.53 | 3.53 | 3.72 | — |
| **D** | *102.3* | *81.3* | *71.3* | *73.8* | *70.4* | — |
| | 5.29 | 4.18 | 3.89 | 3.51 | 3.75 | — |
| **C** | *102.0* | *80.0* | *71.4* | *73.5* | *70.8* | — |
| | 5.09 | 4.06 | 3.92 | 3.48 | 3.87 | — |
| **B-ol** | *64.2* | *74.0* | *80.0* | *76.1* | *68.0* | *20.5* |
| | 3.65, 3.83 | 3.90 | 3.93 | 3.61 | 3.75 | 1.30 |
| | | | OS7 | | | |
| **E** | *105.5* | *74.7* | *76.9* | *73.1* | *77.3* | *174.3* |
| | 4.61 | 3.43 | 3.53 | 3.52 | 3.73 | — |
| **D** | *101.2* | *81.3* | *71.5* | *74.5* | *70.3* | — |
| | 5.21 | 4.08 | 3.89 | 3.45 | 3.88 | — |
| **C-ol** | *62.6* | *80.4* | *69.4* | *74.5* | *68.9* | *20.1* |
| | 3.85 | 3.78 | 3.97 | 3.52 | 3.87 | 1.26 |
| | | | OS8 | | | |
| **B** | *102.6* | *72.0* | *71.5* | *73.2* | *70.2* | — |
| | 4.88 | 3.82 | 3.74 | 3.44 | 3.98 | |
| **A** | *103.2* | *56.9* | *82.6* | *69.7* | *77.0* | *61.9* |
| | 4.77 | 3.87 | 3.65 | 3.54 | 3.47 | 3.77, 3.93 |
| **D** | *100.4* | *71.3* | *81.3* | *72.2* | *70.4* | — |
| | 4.98 | 4.22 | 3.90 | 3.52 | 3.94 | — |
| **C′** | *90.4* | *81.2* | *60.7* | — | — | — |
| | 5.12 | 3.65 | 3.74, 3.87 | — | — | — |

*Notes.* Structures of the CPS and OS1—OS8, as well as the corresponding designations of the monosaccharide residues are given in Fig. 1. $^{13}C$ NMR chemical shifts are italicized. The C(6) and H(6) signals of Rha residues are at $\delta_C$ 17.7—18.1 and $\delta_H$ 1.26—1.32, *N*-acetyl group signals of GlcNAc and GlcNAc-ol residues are at $\delta_C$ 23.3—23.5 (Me) and 175.1—175.5 (CO), $\delta_H$ 2.03—2.06.
* When attached to **A**α; $\delta_{C(1)}$ 102.5 and $\delta_{H(1)}$ 4.85 when attached to **A**β.

**Table 2.** Correlations between the atoms of the adjacent monosaccharide residues in 2D $^1$H—$^1$H ROESY and $^1$H—$^{13}$C HMBC spectra of the CPS from *A. baumannii* strain MAR 55-66 and related OS1—OS8 (see Fig. 1).

| Anomeric/ substituted atoms of adjacent monosaccharide residues | Chemical shifts of the inter-residue correlation peaks (δ/δ) | | |
|---|---|---|---|
| | $^1$H–$^1$H ROESY H(1)/H(X)* | $^1$H—$^{13}$C HMBC C(1)/H(X)* | H(1)/C(X)* |
| | | CPS | |
| G(1)/F(3) | 5.02/3.78 | 103.6/3.78 | 5.02/79.3 |
| F(1)/E(4) | 4.74/3.66 | 102.2/3.66 | 4.74/80.5 |
| E(1)/D(2) | 4.79/4.40 | 104.9/4.40 | 4.79/79.5 |
| D(1)/C(2) | 5.17/4.04 | 102.4/4.04 | 5.17/79.3 |
| C(1)/B(3) | 5.14/3.78 | 102.1/3.78 | 5.14/78.7 |
| B(1)/A(3) | 4.86/3.64 | 102.5/3.64 | 4.86/82.8 |
| A(1)/D(3) | 4.73/3.91 | 103.4/3.91 | 4.73/81.2 |
| | | OS1 | |
| G(1)/F(3) | 5.04/3.80 | 103.5/3.80 | 5.04/79.4 |
| F(1)/E(4) | 4.75/3.61 | 101.7/3.61 | 4.75/80.2 |
| E(1)/D(2) | 4.61/4.16 | 105.5/4.16 | 4.61/81.3 |
| D(1)/C(2) | 5.25/4.05 | 102.3/4.05 | 5.25/79.6 |
| C(1)/B(3) | 5.16/3.77 | 102.0/3.77 | 5.16/78.7 |
| B(1)/Aα(3) | 4.87/3.79 | 102.3/3.79 | 4.87/80.6 |
| B(1)/Aβ(3) | 4.85/3.61 | 102.5/3.61 | 4.85/83.0 |
| | | OS2 | |
| G(1)/F(3) | 5.04/3.80 | 103.6/3.80 | 5.04/79.5 |
| F(1)/E(4) | 4.75/3.62 | 101.8/3.62 | 4.75/80.3 |
| E(1)/D(2) | 4.61/4.16 | 105.6/4.16 | 4.61/81.4 |
| D(1)/C(2) | 5.26/4.06 | 102.4/4.06 | 5.26/79.8 |
| C(1)/B(3) | 5.20/3.84 | 102.1/3.84 | 5.20/78.8 |
| B(1)/A-ol(3) | 4.96/4.08 | 102.7/4.08 | 5.26/78.6 |
| | | OS3 | |
| G(1)/F(3) | 5.04/3.81 | 103.5/3.81 | 5.04/79.4 |
| F(1)/E(4) | 4.75/3.62 | 101.7/3.62 | 4.75/80.2 |
| E(1)/D(2) | 4.61/4.16 | 105.4/4.16 | 4.61/81.3 |
| D(1)/C(2) | 5.26/4.04 | 102.2/4.04 | 5.26/79.8 |
| C(1)/B-ol(3) | 5.08/3.93 | 101.9/3.93 | 5.08/79.8 |
| | | OS4 | |
| G(1)/F(3) | 5.02/3.79 | 103.7/3.79 | 5.02/79.3 |
| F(1)/E(4) | 4.74/3.67 | 102.3/3.63 | 4.74/80.2 |
| E(1)/D(2) | 4.67/4.06 | 105.5/4.06 | 4.67/81.4 |
| D(1)/C-ol(2) | 5.15/3.73 | 101.0/3.73 | 5.15/80.2 |
| | | OS5 | |
| E(1)/D(2) | 4.62/4.18 | 105.4/4.18 | 4.62/81.2 |
| D(1)/C(2) | 5.29/4.10 | 102.1/4.10 | 5.29/79.7 |
| C(1)/B(3) | 5.21/3.87 | 102.0/3.87 | 5.21/79.4 |
| B(1)/A-ol(3) | 4.95/4.29 | 103.4/4.29 | 4.95/77.2 |
| | | OS6 | |
| E(1)/D(2) | 4.63/4.18 | 105.5/4.18 | 4.63/81.3 |
| D(1)/C(2) | 5.29/4.06 | 102.3/4.06 | 5.29/80.0 |
| C(1)/B-ol(3) | 5.09/3.93 | 102.0/3.93 | 5.09/80.0 |
| | | OS7 | |
| E(1)/D(2) | 4.61/4.08 | 105.5/4.08 | 4.6(1)/81.3 |
| D(1)/C-ol(2) | 5.21/3.78 | 101.2/3.78 | 5.21/80.4 |
| | | OS8 | |
| B(1)/A(3) | 4.88/3.65 | 102.6/3.65 | 4.88/82.6 |
| A(1)/D(3) | 4.77/3.90 | 103.2/3.90 | 4.77/81.3 |
| D(1)/C′(2) | 4.98/3.65 | 100.4/3.65 | 4.98/81.2 |

* C(X) is a linked carbon atom, H(X) is a proton at the linked carbon atom.

**Table 3.** ESI HRMS data of the oligosaccharides OS1—OS8 obtained from the CPS of *A. baumanii* strain MAR 55-66

| Oligosaccharide | Monosaccharide composition* | Molecular formula | Ion, *m/z* (experimental value /calculated value) | |
|---|---|---|---|---|
| | | | $[M + Na]^+$ | $[M + K]^+$ |
| OS1 | $R_5A_1N_1$ | $C_{44}H_{73}N_1O_{32}$ | 1150.3955/1150.4008 | 1166.3758/1166.3747 |
| OS2 | $R_5A_1N$-ol | $C_{44}H_{75}N_1O_{32}$ | 1152.4165/1152.4164 | 1168.3837/1168.3904 |
| OS3 | $R_4A_1R$-ol | $C_{36}H_{62}O_{27}$ | 949.3377/949.3371 | 965.3044/965.3110 |
| OS4 | $R_3A_1R$-ol | $C_{30}H_{52}O_{23}$ | 803.2791/803.2792 | 819.2524/819.2531 |
| OS5 | $R_3A_1N$-ol | $C_{32}H_{55}N_1O_{24}$ | 860.3001/860.3006 | 876.2683/876.2746 |
| OS6 | $R_2A_1R$-ol | $C_{24}H_{42}O_{19}$ | 657.2209/657.2213 | 673.1947/673.1952 |
| OS7 | $R_1A_1R$-ol | $C_{18}H_{32}O_{15}$ | 511.1630/511.1633 | 527.1367/527.1373 |
| OS8 | $R_2N_1G_1$ | $C_{23}H_{41}N_1O_{17}$ | 626.2261/626.2267 | 642.2003/642.2006 |

* A is glucuronic acid; G is glyceraldehyde hydrate; N is 2-acetamido-2-deoxyglucose; N-ol is 2-acetamido-2-deoxyglucitol; R is rhamnose; R-ol is rhamnitol.

five or six L-Rha, one D-GlcNAc, and one D-GlcA residues.[2] The corresponding strains of *A. baumannii* also have related gene clusters encoding enzymes for the biosynthesis of the CPSs (these data will be published elsewhere) that shows their origination from each other or a common evolutionary ancestor. The oligosaccharides obtained by

cleavage of *A. baumannii* MAR 55-66 CPS with phage depolymerase may be used for synthesis of conjugated vaccines to fight diseases caused by this bacterium.

## Experimental

**Cultivation of bacteria and isolation of capsular polysaccharide.** *A. baumannii* strain MAR 55-66 was obtained from the collection of the Institute of Antimicrobial Therapy, Smolensk State Medical University. Bacteria were cultivated in a 2TY medium for 16 h, bacterial cells were separated by centrifugation (10000 *g*, 20 min), washed with Na-phosphate buffer (pH 7.5), suspended in a acetone—water mixture (70 : 30 vol.%), precipitated, and dried in air.

Capsular polysaccharide (CPS) was isolated by extractions of cells with aqueous phenol as earlier described.[3] The extract was dialyzed without separation of the layers, insoluble impurities were removed by centrifugation, the solution was acidified with 50% $CCl_3CO_2H$ to pH ~2 at 4 °C. The precipitate was removed by centrifugation (12000 *g*, 20 min), the supernatant was neutralized, concentrated, and fractionated by gel-permeation chromatography on a Sephadex G-50 column (70×2.6 cm, Healthcare) in 0.1% HOAc monitoring the elution with a UV detector (Uvicord, LKB, Sweden) at 206 nm.

**Carbohydrate analysis.** A sample of CPS (2 mg) was hydrolyzed with 3 *M* $CF_3CO_2H$ (120 °C, 2 h). Monosaccharides obtained were identified with a Biotronik LC 2000 carbohydrate analyzer (Germany). Neutral carbohydrates were analyzed by column chromatography (Durrum DA×8 anion exchange resin, 15×0.4 cm) in 0.2 *M* and 0.5 *M* Na-borate buffers (pH 8) at 70 °C. Uronic acid was identified on the same column in 0.02 *M* K-phosphate buffer (pH 3). Amino sugars were determined on a column with Ostion LC ANB cation resin (22×0.4 cm) in 0.2 *M* Na-borate buffer (pH 5).

**Depolymerization of capsular polysaccharide.** Recombinant depolymerase from bacteriophage vB_AbaP_APK86 was cloned, expressed, and purified as described.[4] A CPS sample was dissolved in 20 m*M* Tris-HCl buffer (pH 7.5), depolymerase was added at a volume ratio of the solution protein to CPS of 1 : 100, and the reaction mixture was kept at 37 °C for 16 h. The products of cleavage were fractionated by gel-permeation chromatography on a column 110×1.2 cm of Sephadex G-50 (Amersham Biosciences, Sweden) in water monitoring the elution with a refractive index detector (Knauer, Germany), and after the repeated chromatography oligosaccharides related to the repeating unit of the CPS (OS1), its dimer, and higher oligomers were obtained in a ratio of ca. 2.5 : 2 : 1.

Solvolytic degradation was carried out by adding 99.5% $CF_3CO_2H$ (0.7 mL) to a sample of CPS (12 mg) followed by heating at 45 °C for 20 min, the acid was removed with air flow, the residue was dissolved in water (0.5 mL) and neutralized with concentrated aqueous NaOH. After gel-permeation chromatography as described above, OS1 (4 mg) was obtained.

For the Smith degradation, $NaIO_4$ (43 mg) was added to a solution of CPS (18 mg) in water (2 mL). The solution was kept in dark at 20 °C for 40 h, and $NaBH_4$ (70 mg) was added portionwise, after 2 h excess $NaBH_4$ was quenched with HOAc, boronic acid was removed by triple coevaporation with MeOH and HOAc, and the residue was deionized by gel-permeation chromatography on Sephadex G-25 as described above. The resulting modified CPS (15 mg) was hydrolyzed with 2% HOAc (110 °C, 2 h), the products were fractionated on the same column, and from the major (trisaccharide) fraction (7 mg) OS8 (2.5 mg) was obtained by reversed-phase HPLC on a Zorbax C18 column (25×1 cm) eluated with water.

**Chemical modifications of OS1.** To a solution of OS1 (13 mg) in water (1 mL), $NaBH_4$ (15 mg) was added portionwise, the solution was kept at 20 °C for 16 h, excess $NaBH_4$ was quenched with HOAc, boronic acid was removed by coevaporation with MeOH and HOAc. The residue was subjected to gel-permeation chromatography on Sephadex G-25 as described above to isolate OS2 (10 mg).

The OS1 sample (8 mg) was dissolved in aqueous 0.1 *M* NaOH (0.5 mL), the solution was kept at 40 °C for 1 h, neutralized to pH 7—8, and $NaBH_4$ (20 mg) was added. The mixture was kept at 20 °C for 16 h and worked up as described above to give OS3 (4 mg). A similar treatment of OS1 (13 mg) under more drastic conditions (0.5 *M* NaOH, 50 °C, 2 h) afforded OS4 (6 mg).

Samples of OS2, OS3, or OS4 (4—7 mg each) were heated with 0.2 *M* $CF_3CO_2H$ (1 mL) at 100 °C for 1 h, the solution was concentrated, and gel-permeation chromatography of the residues on Sephadex G-25 as described above afforded OS5, OS6, or OS7 (2—3 mg each), respectively.

**NMR spectroscopy.** For NMR analysis, the samples were lyophilized from 99.9% $D_2O$ and dissolved in 99.95% $D_2O$. NMR spectra were registered with an Avance II instrument (600 MHz, Bruker, Germany) at either 40 °C for CPS and OS8 or 50 °C for OS1—OS7 using sodium 3-trimethylsilylpropanoate-2,2,3,3-$d_4$ ($\delta_H$ 0.0, $\delta_C$ −1.6) as an internal standard. Two-dimensional $^1H—^1H$ correlation spectroscopy (COSY and TOCSY), $^1H—^1H$ rotating frame nuclear Overhauser effect spectroscopy (ROESY), heteronuclear single-quantum (HSQC) and multiple bond $^1H—^{13}C$ correlation (HMBC) spectroscopy were carried out using pulse sequences (Bruker, Germany). TopSpin 2.1 software (Bruker, Germany) was used for data acquisition and data processing. Spin stabilization MLEV-7 time and mixing time in TOCSY and ROESY experiments were 60 and 200 ms, respectively. For optimization of the $^1H—^{13}C$ HMBC experiments, a 60 ms delay that corresponded to the spin coupling constant $J_{H,C}$ = 8 Hz was used for development of the multibond correlations.

**Mass spectrometry.** High-resolution electrospray ionization mass spectrometry (ESI HRMS) was used for analysis of oligosaccharides in the positive ion mode using micrOTOF II and maXis (Bruker Daltonics) instruments.[8] Samples (ca. 50 ng $\mu L^{-1}$) were dissolved in water—acetonitrile (50 : 50 vol.%) and injected by a syringe into an ion source at a flow rate of 3 $\mu L\ min^{-1}$ using nitrogen as the nebulizer gas (4 $L\ min^{-1}$). Capillary voltage was set at −4500 V, interface temperature was set at 180 °C, and acquisition range was *m/z* 50—3000. Electrospray Calibrant Solution (Agilent Technologies, USA) was used for internal calibration.

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