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ARTICLE

# Synthesis and evaluation of biological activity of benzoxaborole derivatives of azithromycin

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## Abstract

Novel benzoxaborole derivatives of azithromycin in which benzoxaborole residue is attached to the 4″-hydroxy-group of azithromycin have been synthesized. Antibacterial activity of synthesized derivatives in comparison with azithromycin was tested on a panel of Gram-positive and Gram-negative bacterial strains. All the investigated compounds demonstrated broad spectrum of antibacterial activity being in general more active against Gram-positive strains. New benzoxaborole derivatives of azithromycin demonstrated high activity against *Streptococcus pyogenes* ATCC 19615 and *Propionibacterium acnes* ATCC 6919 strains. Some of the new compounds were more active than azithromycin against *Streptococcus pneumoniae* ATCC 49619 strain or *Enterococcus faecium* strains. Using a reporter construct created on the basis of the transcription attenuator region of the *Escherichia coli* tryptophan operon pRFPCER-TrpL2A it has been demonstrated that the mechanism of action of azithromycin analogs is blocking nascent peptide in ribosome tunnel.

## Introduction

Concept of hybrid antibiotics is one of the developing strategies for the search of new drugs which can overcome multidrug resistance of bacteria, have a broader spectrum of action compared with the initial antibiotics, and retard the development of antibiotic resistance [1, 2]. Azithromycin (**1**) has served as a scaffold for different series of dual-acting antibacterials, including large number of azithromycin–fluoroquinolones hybrids (macrolones) described by GlaxoSmithKline and Pliva [3–10].

The mechanism of action of macrolides is related to the protein synthesis inhibition in the microbial cell upon interaction of the antibiotic with the V-domain of 23S rRNA in the peptidyl transferase site of a ribosome. The mechanisms of resistance to macrolides are connected with the active efflux of the antibiotic out of the cell and the target modification via specific post-transcriptional modification (methylation) of 23S rRNA or mutations of the genes encoding either 23S rRNA or ribosomal proteins [11].

Anacor Pharmaceuticals (acquired by Pfizer in 2016) has developed a technology based on the use of boron chemistry to develop novel therapies including antimicrobials. Boron has two attributes that may provide compounds with drug-like properties — unique geometry that allows boron-based compounds interact with biological targets in novel ways and increased reactivity as compared to carbon that allows designing molecules that can hit targets that are difficult to inhibit with carbon chemistry [12, 13]. Based on this approach Anacor has developed Kerydin®, benzoxaborole-based drug, which has been approved by FDA for treatment onychomycosis in 2014 and crisaborole which has been approved by FDA in the end of 2016 for the treatment of mild-to-moderate atopic dermatitis.

Moreover, the benzoxaboroles were successfully used to obtain new types of hybrid molecules, such as benzoxaborole-glycopeptides conjugates which have demonstrated activity against Gram-positive bacteria including the resistant isolates *Staphylococci* GISA and *Enterococci* GRE [14]. A novel group of benzoxaborole-

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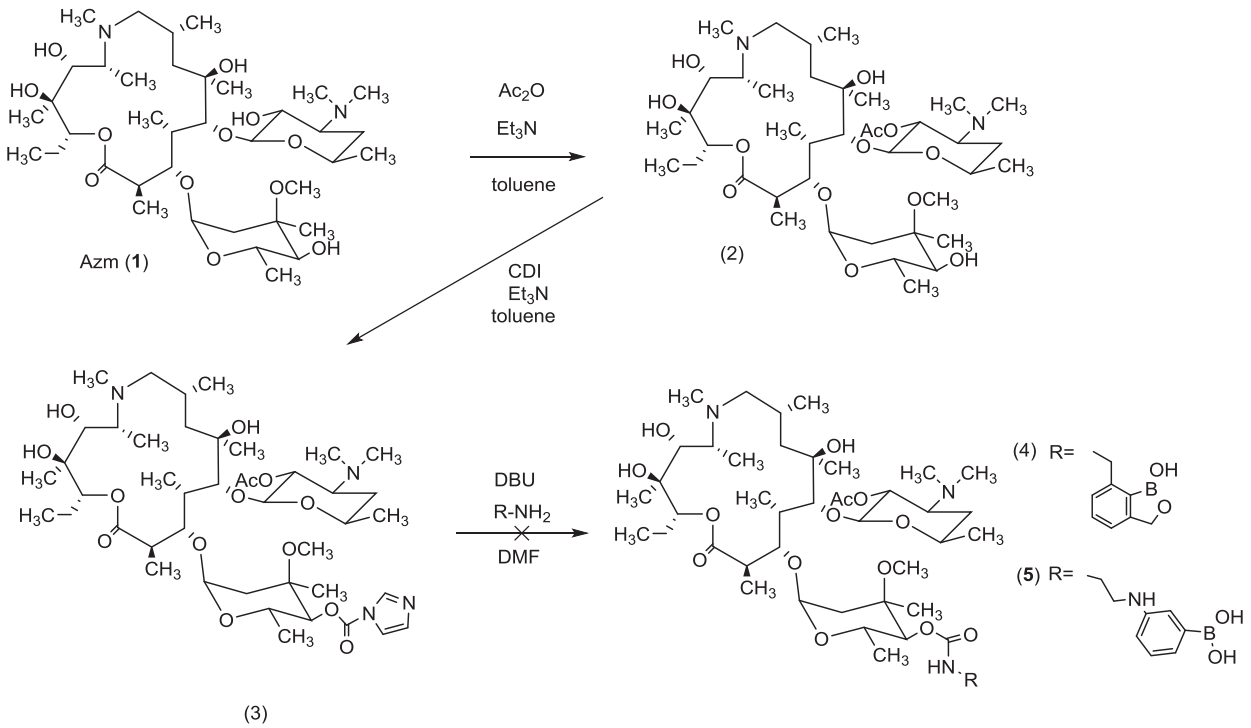



**Scheme 1** Synthesis of borone-containing derivatives of azithromycin by direct reaction of amino-containing benzoxaborole or boronic acid with the activated intermediate of azithromycin

chalcone hybrids highly potent against parasites *Trypanosoma brucei* has been described [15]. Recently, amphotericin B-benzoxaborole conjugates with lower hemolytic toxicity were described [16]. Previously, in our group, benzo[c] [1, 2]oxaboroles were used as active fragments for synthetic transformation of clarithromycin resulting in new semisynthetic antibiotics that were active only against Gram-positive strains [17].

Herein we report synthesis and evaluation of biological activity of novel benzoxaborole derivatives of azithromycin in which benzoxaborole residue is attached to the 4″-*O*-group of azithromycin.

## Results and discussion

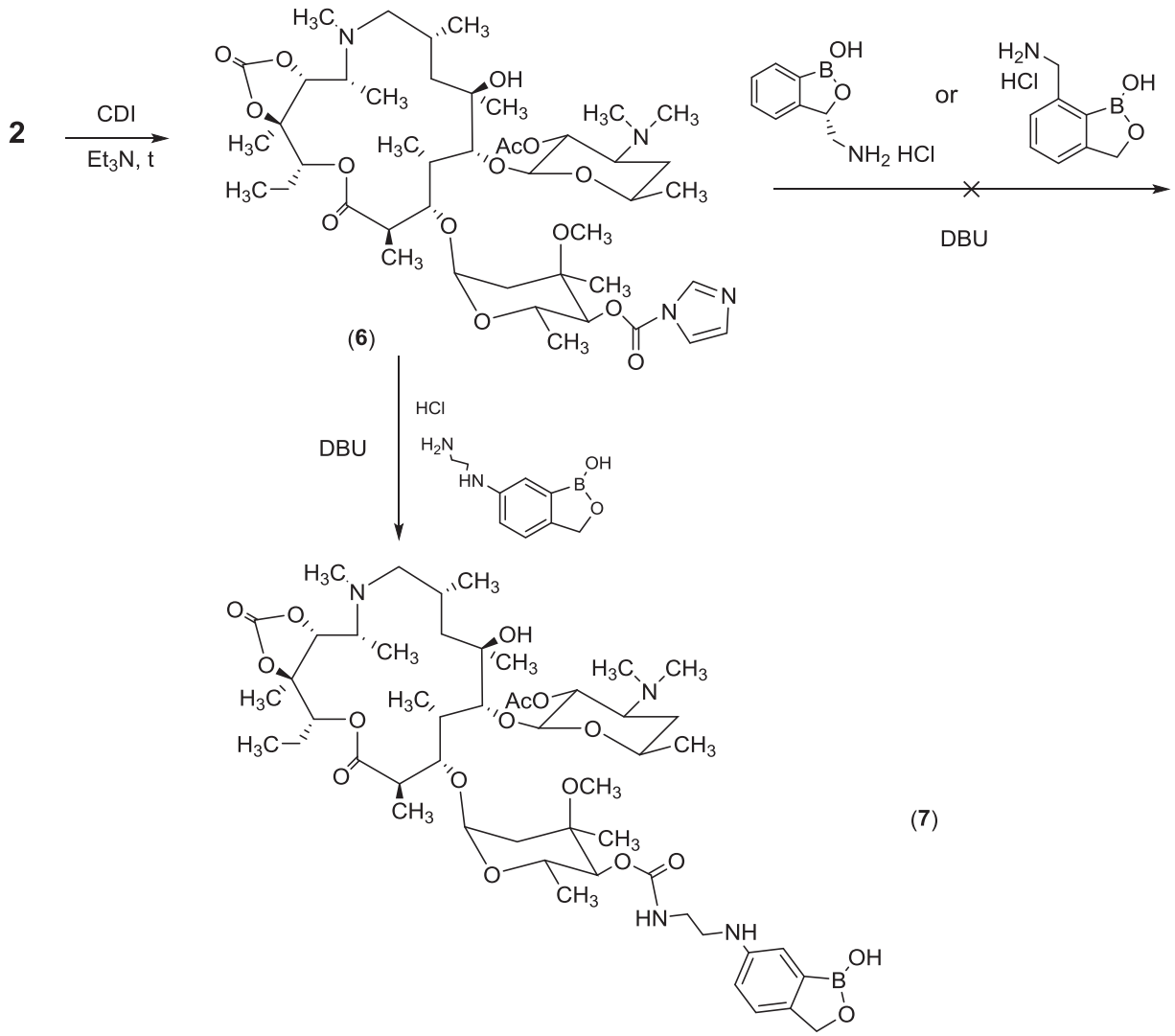
### Chemistry

First, we tried to obtain 4″-*O*-benzoxoborolcarbamoyl derivatives of azithromycin by direct reaction of benzoxaboroles with 2′-*O*-acetylazithromycin (**2**) (Scheme 1). The 2′-*O*-hydroxyl group of azithromycin was protected with the acetyl group; 4″-*O*-hydroxyl group of 2′-*O*-acetylazithromycin (**2**) was activated by the reaction with carbonyldiimidazole (CDI) in the presence of $Et_3N$. The reaction of the intermediate **3** with benzoxaborole or boronic acid which contain the amino group was carried out in the presence of DBU (1,8-diazabicyclo[5.4.0]undec-7-ene) but the reaction accompanied by the formation of significant amounts of by-products, and even the attempts to purify the target compounds **4** and **5** using the semipreparative high-performance liquid chromatography (HPLC) method were unsuccessful.

Next step was use of method described in ref. [18] for the synthesis of the protected and activated derivative of azithromycin-2′-*O*-acetyl-4″-*O*-acylimidazolylazithromycin 11,12-cyclic carbonate (**6**) (Scheme 2), which was further used for the reaction with the amino-containing benzoxaboroles.

Unfortunately, as in the previous case, the reaction of the intermediate **6** with benzoxaboroles containing the amino group proceeded very slow and with the formation of the big amount of by-products (Scheme 2). Although we were able to obtain the derivative **7** by the reaction of compound **6** with the corresponding benzoxaborole after purification by the column chromatography on silica gel, on the whole the method was not suitable for the synthesis of a series of azithromycin–benzoxaborole derivatives. The attempt of the removal of the protecting 2′-*O*-acetyl group of **7** in MeOH led to a significant decrease in purity of the obtained compound, and we were not able to purify them by column chromatography methods.

The following step on a way to the synthesis of benzoxaborole derivatives of azithromycin was the introduction of a spacer between the azithromycin and benzoxaborole molecules. The use of the diamino alkyl spacer allowed



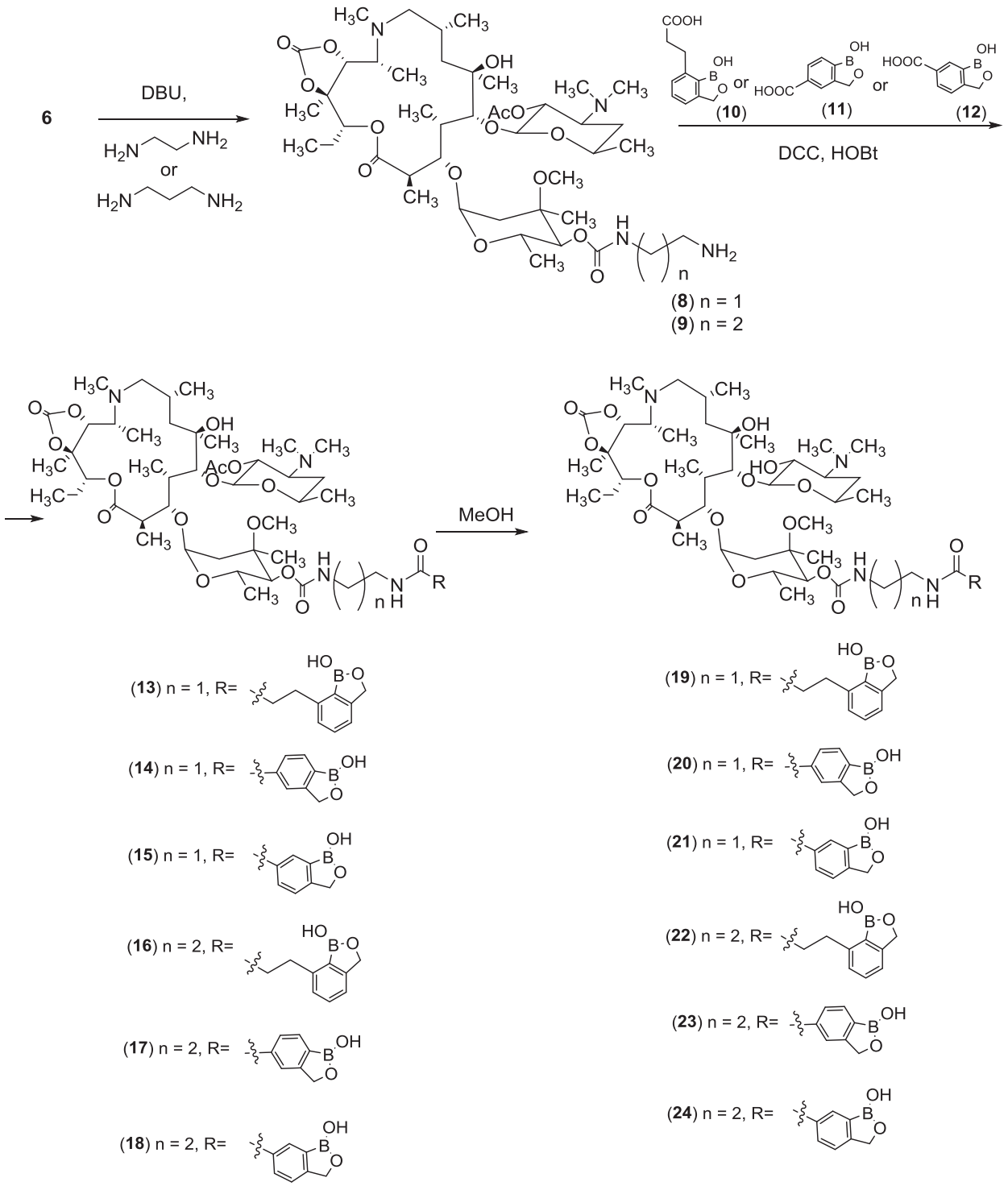

**Scheme 2** Reaction of 2′-*O*-acetyl-4″-*O*-acylimidazolylazithromycin 11,12-cyclic carbonate (**6**) with amino-benzoxaboroles

using carboxy-containing benzoxaboroles for the conjugation with azithromycin (Scheme 3).

The reaction of the intermediate **6** with ethylenediamine or propylenediamine proceeded smoothly in the presence of DBU, the obtained protected azithromycin derivatives containing the amino group (**8** or **9**) were acylated by the benzoxaboroles containing the carboxylic group (**10**, **11** or **12**) in the presence of DCC (*N*,*N*′-dicyclohexylcarbodiimide) and HOBt (hydroxybenzotriazole) (Scheme 3). The resulting derivatives **13–18** were purified by flash chromatography on silica gel. The acetyl group was removed by keeping the protected compounds **13–18** in MeOH at 37 °C for 24 h resulting in the 2′-unprotected compounds **19–24**. The attempts to remove the 11,12-cyclic carbonate group from compounds **19–24** using the method described in ref. [6] (MeOH/$H_2O$ 2:1, $K_2CO_3$ 15 equiv., 55 °C, 2 h) were unsuccessful because of the formation of significant amounts of side products, one of them is supposed to be a methylated derivatives (judging from ESI mass spectra data).

So, antibiotics **29**, **30** without the 11,12-cyclic carbonate group were obtained starting from 2′-*O*-acetyl-4″-*O*-acylimidazolylazithromycin (**3**) (Scheme 4).

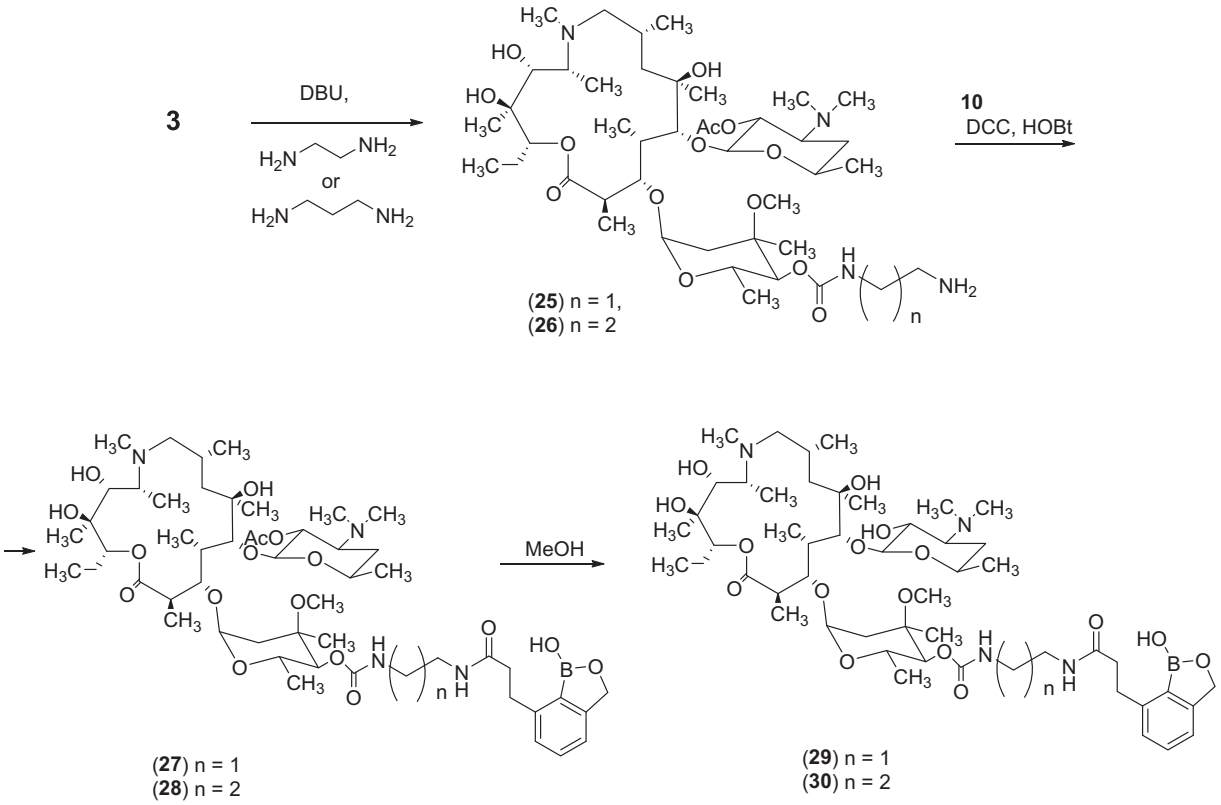
The reaction of the activated intermediate **3** with ethylenediamine or propylenediamine was carried out in the presence of DBU, and the obtained azithromycin derivatives containing the amino group (**25** or **26**) were acylated by the benzoxaborole **10** in the presence of DCC and HOBt (Scheme 4). The resulting derivatives **27, 28** were purified by flash chromatography on silica gel. The acetyl group was removed by keeping the 2′-*O*-acetyl derivatives **27, 28** in MeOH at 37 °C for 24 h. Unfortunately, it was much more difficult to purify compounds **29, 30** than the corresponding derivatives **19–24** which contain 11,12-cyclic carbonate




**Scheme 3** Synthesis of benzoxaborole derivatives of azithromycin

group. So the yields in the case of synthesis of derivatives **29, 30** using intermediates which did not contain the 11,12-cyclic carbonate group were low.

Purity of the obtained compounds **7**, **13–24**, **29**, **30** was confirmed by the HPLC method. Structures of the obtained compounds **7**, **13–24**, **29**, **30** were confirmed using mass spectrometry and NMR spectroscopy methods. $^{1}H$ and $^{13}C$ NMR spectra of derivatives **7**, **13–24**, **29**, **30** contained all the signals corresponding to the benzoxaborole and azithromycin parts of the molecule as well as the signals of carbon atoms of the spacers. Although we were able to identify all characteristic signals in the NMR spectra




**Scheme 4** Synthesis of azithromycin derivatives **29, 30** without the cyclic carbonate group

(signals of benzoxaborole moieties, acetyl, methoxy, methyl groups, carbonyl), total assignment of NMR spectra was complicated by the existing dynamic equilibrium in the solutions of the studied benzoxaborole derivatives of azithromycin. The signals of aromatic residues of benzoxaborole moieties were easily assigned and appeared at 6.9–7.9 ppm in the $^{1}$H NMR spectra and at 114–160 ppm in the $^{13}$C NMR spectra of compounds **7**, **13–24**, **29**, **30**. Signals of the amide carbon atoms (for all compounds) and carbonate carbon atom (in the case of compounds **7**, **13–24**) appeared at 169–182 ppm in the corresponding $^{13}$C NMR spectra. Mass spectra of the obtained compounds **7**, **13–24**, **29**, **30** by the HRMS with electrospray ionization contained the signals corresponding to the molecular ion $(M + H)^{+}$ and $(M + H)^{2+}$.

Antibacterial activity of synthesized derivatives **7**, **13–24**, **29**, and **30** in comparison with that of azithromycin (**1**) was tested on a panel of Gram-positive and Gram-negative bacterial strains (Table 1). All the investigated compounds demonstrated broad spectrum of antibacterial activity being more or less active against both Gram-positive and Gram-negative bacterial strains, although in general introduction of the benzoxaborole moiety significantly reduced activity of the obtained derivatives against Gram-negative bacteria in comparison with **1**.

Benzoxaborole derivatives of azithromycin **7**, **13–24** demonstrated high activity against *S. pyogenes* ATCC 19615 and *P. acnes* ATCC 6919 strains. Interestingly, compounds **14**, **20**, **22**, and **24** were more active (MICs 0.13 μg ml$^{-1}$) that azithromycin against *S. pneumoniae* ATCC 49619 strain (MIC 4 μg ml$^{-1}$). Azithromycin derivatives **7**, **14–22**, and **24** were more active (MICs 0.13–2 μg ml$^{-1}$) than **1** (MIC 8 μg ml$^{-1}$) against *E. faecium* 568 strain. Compounds **7**, **19**, and **22** also were more active (MICs 1–2 μg ml$^{-1}$) that azithromycin (**1**) (MIC 8 μg ml$^{-1}$) against *E. faecium* 569 strain.

On the whole, the presence of the 2′-*O*-acetyl or 11,12-cyclic carbonate groups seems do not influence significantly to the antibacterial activity of the studied compounds. Interestingly, the longer spacer between the benzoxaborole moiety and azithromycin (compounds **19**, **22**, **29**, and **30**) seems to be preferable for the activity against Gram-negative strains (Table 1) while the cyclic carbonate is favorable for the high activity of the studied compounds against *S. pneumoniae* ATCC 49619 and *E. faecium* strains (compounds **7**, **14**, **19**, **20**, **22**, **24** vs. compounds **29** and **30**).

Using a reporter construct created on the basis of the transcription attenuator region of the *Escherichia coli* tryptophan operon pRFPCER-TrpL2A [19] reporter strain, the mechanism of action of azithromycin analogs **13**, **14**,

**Table 1** Antibacterial activity of benzoxaborole derivatives of azithromycin in comparison with that of azithromycin (**1**) against a panel of Gram-positive and Gram-negative strains

| Strain | Compound, MIC, μg ml$^{-1}$ | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2′-*O*-acetyl-11,12-cyclic carbonate | | | | | | | 11,12-cyclic carbonate | | | | | | 11,12-OH | |
| | | 7 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 29 | 30 |
| **Gram positive** | | | | | | | | | | | | | | | | |
| *St. aureus* ATCC 25923 | 1 | 16 | 32 | 16 | nt | nt | nt | nt | 16 | 32 | nt | 4 | nt | 16 | 32 | 8 |
| *St. aureus* 10 | 1 | nt | 16 | 2 | nt | nt | nt | nt | nt | 2 | nt | 0.5 | nt | 1 | 16 | 16 |
| *St. epidermidis* ATCC 12228 | 0.5 | 2 | 8 | 64 | 32 | 8 | 32 | 32 | 8 | 32 | 32 | 8 | 32 | 32 | 8 | 4 |
| *S. pyogenes* ATCC 19615 | ≤0.12 | ≤0.12 | 0.25 | 2 | 1 | 0.25 | 2 | 0.5 | 0.25 | 1 | 1 | 0.25 | 1 | 0.5 | nt | nt |
| *S. pneumoniae* ATCC 49619 | 4 | nt | 16 | 0.13 | nt | nt | nt | nt | nt | 0.13 | nt | 0.13 | nt | 0.13 | 16 | 16 |
| *E. faecium* 568 | 8 | 2 | 16 | 0.13 | 2 | 2 | 0.13 | 2 | 2 | 0.13 | 2 | 0.13 | nt | 0.13 | 16 | 8 |
| *E. faecium* 569 | 8 | 2 | 16 | >32 | nt | nt | nt | nt | 1 | >32 | nt | 2 | nt | 32 | 16 | 16 |
| *P. acnes* ATCC 6919 | ≤0.12 | 0.25 | ≤0.12 | 1 | 1 | ≤0.12 | 0.5 | 0.5 | 0.25 | 0.5 | 0.5 | ≤0.12 | 0.5 | 0.5 | nt | nt |
| **Gram negative** | | | | | | | | | | | | | | | | |
| *E.coli* ECM 1888 | 0.5 | 4 | 16 | 32 | 32 | 16 | 32 | 32 | 8 | 16 | 32 | 8 | 32 | 32 | 8 | 4 |
| *E. coli* leuS ANA395 | 0.5 | 4 | 16 | 32 | 32 | 16 | 32 | 32 | 16 | 32 | 32 | 8 | 32 | 32 | 4 | 4 |
| *P. multocida* ATCC 11039 | 0.25 | 2 | 8 | 32 | 16 | 8 | 32 | 32 | 4 | 16 | 16 | 4 | 32 | 16 | 4 | 4 |
| *M. haemolytica* ATCC 33396 | 1 | 4 | 8 | 64 | 64 | 16 | 64 | 64 | 16 | 32 | 32 | 8 | 64 | 32 | 16 | 32 |
| *H. somni* ATCC 700025 | ≤0.12 | 4 | 8 | 32 | 16 | 8 | 16 | 16 | 4 | 16 | 16 | 4 | 16 | 16 | 4 | 4 |

**16–24**, **29**, and **30** was tested. All the tested compounds **13**, **14**, **16–24**, **29**, and **30** induced reporter as erythromycin, so mechanism of action is the same — blocking nascent peptide in ribosome tunnel. The size of inhibition zone indicates the efficiency of the antibiotic on this model (Fig. 1). Only one of the tested benzoxaboroles, compound **10**, demonstrated the ability to induce pRFPCER-TrpL2A reporter (Fig. 1).

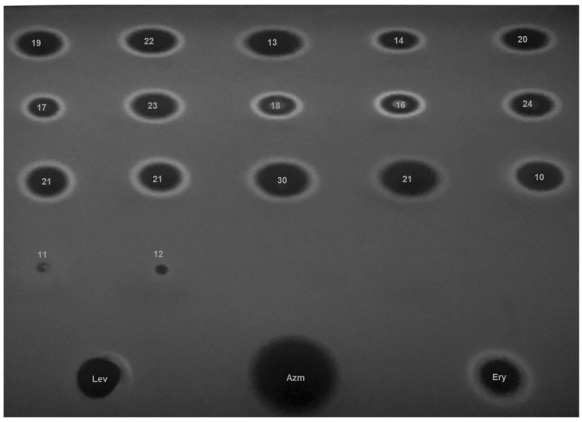



**Fig. 1** Induction of translation inhibitor reporter by azithromycin and its new derivatives. The lawn of *E. coli* ΔtolC transformed with pRFPCER-TrpL2A was spotted on agar. Circles of the Cerulean protein were formed under the influence of antibiotic causing ribosome stalling. Petri dishes were illuminated at UV (254 nm) and documented by a digital camera. Erythromycin (Ery), azithromycin (Azm), and levofloxacin (Lev) were used as positive and negative controls, consequently

## Conclusion

A series of benzoxaborole derivatives of azithromycin have been synthesized in which benzoxaborole residue was attached via spacer to the 4″-hydroxy-group of antibiotic. New azithromycin derivatives demonstrated high activity against *S. pyogenes* ATCC 19615 and *P. acnes* ATCC 6919 strains. Some of the novel compounds were more active than azithromycin against *S. pneumoniae* ATCC 49619 and *E. faecium* strains. The longer spacer between benzoxaborole moiety and azithromycin moieties was preferable for the activity against Gram-negative strains, while the 11,12-cyclic carbonate group was favorable for the high activity of the studied compounds against *S. pneumoniae* ATCC 49619 and *E. faecium* strains. Newly synthesized benzoxaborole derivatives of azithromycin retained the ability of azithromycin to block the nascent peptide in ribosome tunnel.

## Experimental

### General

All necessary solvents were purified prior to use, unless noted otherwise. Reactions were monitored by thin-layer chromatography (TLC) using Merck Silica Gel 60F254

plates. Flash column chromatography was performed with the indicated solvents using Merck silica gel 60. Infrared spectra were obtained on a Nicolet-iS10 Fourier transform IR spectrometer (DTGS detector, splitter—KBr) with a Smart Performer module equipped with a ZnSe-crystal. The spectra were run on the range of 3000–650 $cm^{-1}$ with a resolution of 4 $cm^{-1}$. The spectra were proceeded using the OMNIC-7.0 program package. $^{1}H$ and $^{13}C$ NMR spectra were recorded on a Varian VXR-400 NMR spectrometer (Varian, Palo Alto) at ambient temperature (TMS was used as the internal standard of chemical shifts). High-resolution electrospray mass spectra were recorded on a Bruker «micrOTOF-Q II»-MS instrument (Bruker Daltonics GmbH, Bremen, Germany). The samples were dissolved in mixture AcCN–$H_2O$ (3:2) and analyzed via continuous flow injection at 3 µl $min^{-1}$. The mass spectrometer was operated in positive ion mode with a capillary voltage of 4 kV, an endplate offset of −500 V, nebulizer pressure of 0.4 bar, and a drying gas flow rate of 4 l $min^{-1}$ at 180 °C. The instrument was calibrated with a Fluka electrospray calibration solution (Sigma-Aldrich, Buchi, Switzerland) that was 100 times diluted with acetonitrile. The accuracy was better than 0.43 ppm in a mass range between *m/z* 118.0862 and 2721.8948. All solvents used were purchased in best LC-MS qualities. Analytical reverse phase HPLC was carried out on a Shimadzu HPLC instrument of the LC 10 series at a Kromasil C-18 column (4.6 × 250 mm) at an injection volume of 20 µl using a variable UV detector with flow rate 1.0 ml $min^{-1}$. All systems consisted of buffers — 0.2% $HCOONH_4$ at pH 4.2 — and organic phase — acetonitrile. The proportion of acetonitrile was varied: system A: 20 → 80% for 30 min; system B: 30 → 70% for 30 min.

System A: Elution: A—$HCOONH_4$ 0.2% pH 4.2, B—AcCN, gradient of AcCN from 20 to 80% from 0 to 30 min. System B: Elution: A—$HCOONH_4$ 0.2% pH 4.2, B—AcCN, gradient of AcCN from 30 to 70% from 0 to 30 min.

### 2′-*O*-Acetylazithromycin (2)

To a solution of azithromycin (**1**) (2.0 g, 2.67 mmol) in dichloromethane (20 ml) at room temperature was added acetic anhydride (0.5 ml, 5.34 mmol) and $Et_3N$ (1.48 ml, 10.68 mmol). The resulting solution was allowed to stir for 24 h at the same temperature. The reaction was quenched with 5% aqueous $NaHCO_3$ (20 ml) and the aqueous layer was extracted with dichloromethane (3 × 10 ml). The combined organic layers were dried over anhydrous $Na_2SO_4$, filtered, and concentrated in vacuum. The residue was crystallized from acetone:water (2:1) to afford 1.84 g (92%) of **2** as a white solid: mp 162–166 °C (lit. 167–170 °C [8]); $R_f$ 0.50 (dichloromethane/methanol, 10:1); MS (ESI) *m/z* calcd. For $C_{40}H_{74}N_2O_{13}$ 790.5191; found $(M + H^+)$ 791.4593.

### 2′-*O*-Acetyl-4″-*O*-acylimidazolylazithromycin (3)

To a solution of **2** (1 g, 1.27 mmol) in toluene (15 ml) was added $Et_3N$ (0.45 ml, 2.87 mmol) and CDI (0.66 g, 3.80 mmol). The resulting solution was stirred at rt for 24 h. The reaction was quenched with saturated $NaHCO_3$ (30 ml) and the aqueous layer was extracted with toluene (3 × 10 ml). The combined organic layers were dried over anhydrous $Na_2SO_4$, filtered, and concentrated in vacuum. The residue was purified by flash column chromatography (dichloromethane/methanol, 10:1) to afford 0.92 g (80%) of **3** as a white foam; $R_f = 0.41$ (dichloromethane/methanol, 10:1); MS (ESI) *m/z* calcd. for $C_{44}H_{76}N_4O_{14}$ 884.5358; found $(M + H^+)$ 885.5421.

### 2′-*O*-Acetyl-4″-*O*-acylimidazolylazithromycin 11,12-cyclic carbonate (6)

To a solution of **2** (1.5 g, 1.90 mmol) in toluene (20 ml) was added $Et_3N$ (0.68 ml, 4.33 mmol) and CDI (1.23 g, 7.60 mmol). The resulting solution was heated to 55 °C and stirred at the same temperature for 24 h. The reaction was quenched with saturated $NaHCO_3$ (40 ml) and the aqueous layer was extracted with toluene (3 × 10 ml). The combined organic layers were dried over anhydrous $Na_2SO_4$, filtered, and concentrated in vacuum. The residue was purified by flash column chromatography (dichloromethane/methanol, 20:1) to afford 1.61 g (93%) of **6** as a white solid: mp 114–117 °C (lit. 117–120 °C [8]); $R_f = 0.61$ (dichloromethane/methanol, 10:1); MS (ESI) *m/z* calcd. for $C_{45}H_{74}N_4O_{15}$ 910.5151; found $(M + H^+)$ 911.4505.

### 2′-*O*-Acetyl-4″-*O*-benzoxoborolcarbamoyl azithromycin 11,12-cyclic carbonate (7)

White solid, yield 15%. To a solution of **6** (1.5 g, 1.70 mmol) in DMF (15 ml) was added DBU (0.33 ml, 2.25 mmol) and benzoxaborole AN 9032 (4.5 mmol). The resulting solution was stirred for 24 h at rt. The reaction was quenched with water (15 ml) and the aqueous layer was extracted with ethyl acetate (2 × 15 ml). The combined organic layers were washed with brine, dried over anhydrous $Na_2SO_4$, and filtered. The filtrate was concentrated in vacuo to afford a crude product **7** which was purified by column chromatography on silica gel. The column was pre-equilibrated with $CHCl_3$, the elution was performed with $CHCl_3$ (70 ml), then with the mixture $CHCl_3$-EtOH (10:1) (150 ml), and then with the mixture $CHCl_3$-EtOH (5:1). Fractions that contain the desired product were combined

and evaporated in vacuo to give 120 mg (7%) of the target compound **7** as white solid.

Rt (A) 29.39 min. IR: 3391, 2972, 2936, 2875, 1810, 1739, 1616, 1600, 1575, 1513, 1453, 1377, 1351, 1317, 1236, 1166, 1124, 1085, 1042, 1012, 986, 957, 934, 904, 881, 805, 773, 755, 727. $^{1}$H NMR ($CDCl_3$, δ ppm): 7.77–7.16 (m, 3H); 5.58 (s, 1H); 5.06 (m, 1H); 4.86–4.56 (m, 2H); 4.42 (s, 1H); 4.38 (s, 1H), 4.29 (m, 1H); 3.50 (d, 1H); 3.33–3.26 (m, 3H); 2.42–2.26 (m, 6H), 2.21–2.15 (m, 3H); 2.05 (s, 3H); 1.96–1.76 (m, 3H); 1.67–1.50 (m, 3H), 1.43 (s, 3H), 1.35–1.01 (m, 13H); 0.99–0.84 (m, 6H). $^{13}$C NMR ($CDCl_3$, δ ppm): 180.20, 178.20, 170.11, 158.65, 153.47, 130.99, 125.65, 120.77, 114.60, 100.66, 94.76, 86.22, 84.77, 80.02, 76.45, 71.17, 67.57, 63.09, 60.993, 53.70, 49.32, 40.29, 34.97, 34.54, 30.50, 29.07, 26.57, 26.01, 21.88, 21.15, 20.87, 17.59, 13.69, 10.33, 5.08. MS (ESI) *m/z* cacl. for $C_{51}H_{83}BN_4O_{17}$ 1034.5846; found $(M + H)^+$ 1035.5898 $(M + H)^{2+}$ 518.2937.

## General methods for 4″-*O*-aminoalkylcarbomoyl azithromycin intermediates 8, 9, 25, 26

To a solution of activated imidazolyl derivative **6** (1.5 g, 1.65 mmol) or **3** (1.3 g, 1.65 mmol) in DMF (15 ml) was added DBU (0.33 ml, 2.25 mmol) and ethylenediamine or propylenediamine (3.3 mmol). The resulting solution was stirred for 2 h at room temperature. The reaction was quenched with 5% aqueous $NaHCO_3$ (20 ml) and the aqueous layer was extracted with ethyl acetate (2 × 15 ml). The combined organic layers were washed with brine, dried over anhydrous $Na_2SO_4$, and filtered. The filtrate was concentrated in vacuum to afford a crude product **8, 9** (in the case of starting compound **6**) or **25, 26** (in the case of starting compound **3**) (yields around 90%), which were used in the next stage without additional purification.

## General methods for compounds 13–18, 27, 28

To a solution of the benzoxaborole **10**, **11** or **12** and 1-hydroxy-benzo-triazole (HOBt, 1.65 mmol) in THF (15 ml) was added 1,3-dicyclohexylcarbodiimide (DCC, 1.65 mmol) at 0 °C and reaction was stirred for 1 h at the same temperature. The resulting precipitate was filtered off and the filtrate was added to a solution of 4″-*O*-aminocarbamate (**8** or **9** or **25** or **26**) (1.50 mmol) and 1-hydroxy-benzo-triazole (HOBt, 1.65 mmol) in THF (15 ml) at 0 °C. The reaction mixture was stirred for 1 h at the same temperature and for 12 h at room temperature. The reaction was concentrated in vacuum and ethyl acetate (15 ml) was added. After stirred for 1 h, the insoluble substance was leached. The filtrate was quenched with 5% aqueous $NaHCO_3$ (20 ml) and the aqueous layer was extracted with ethyl acetate (2 × 15 ml). The combined organic layers were washed with brine, dried over anhydrous $Na_2SO_4$, and filtered. The filtrate was concentrated in vacuum to afford the crude products **13–18, 27, 28**, which were further purified by flash column chromatography (dicholoromethane–ethanol, 5:1).

### Compound 13

White solid, yield 24%. Rt (A) 22.98 min. IR: 3357, 2973, 2938, 2879, 1812, 1739, 1657, 1600, 1583, 1531, 1454, 1372, 1167, 1123, 1045, 1014, 985, 957, 904, 831, 804, 775, 737. $^{1}$H NMR ($CDCl_3$, δ ppm): 7.36 (t, 1H); 7.18 (d, 1H), 7.12 (d, 1H); 6.63 (s. 1H); 5.66 (s, 1H); 5.07 (d, 1H); 4.88 (dd, 1H), 4.62 (d, 1H); 4.52 (d, 1H); 4.44 (s, 1H); 4.38 (s, 1H), 4.30 (m, 1H); 3.91 (m, 1H); 3.54 (d, 1H); 3.37 (s, 3H); 3.34–3.27 (m, 2H); 3.16–3.09 (m, 2H); 2.94–2.89 (m, 3H); 2.86–2.81 (m, 2H), 2.43 (s, 1H); 2.40–2.37 (m, 2H); 2.35 (s, 3H); 2.31–2.24 (m, 1H); 2.31 (s, 3H); 2.17 (m, 3H); 2.07 (s, 3H); 1.99–1.79 (m, 4H); 1.67–1.53 (m, 3H), 1.44 (s, 3H), 1.38 (m, 2H); 1.28–1.14 (m, 15H); 1.05 (d, 3H); 0.97–0.87 (m, 7H). $^{13}$C NMR ($CDCl_3$, δ ppm): 177.38, 174.38, 170.09, 157.08, 154.95, 153.58, 145.63, 131.35, 127.26, 119.41, 115.17, 100.20, 95.04, 86.60, 85.00, 79.44, 76.55, 71.93, 70.66, 67.92, 63.36, 61.68, 54.05, 49.68, 45.09, 41.79, 40.66, 40.39, 39.53, 35.35, 34.69, 31.14, 29.47, 27.14, 26.41, 22.35, 18.14, 13.99, 10.68, 5.40. MS (ESI) *m/z* cacl. for $C_{54}H_{87}BN_4O_{18}$ 1090.6108 found $(M + H)^+$ 1091.6298, $(M + H)^{2+}$ 546.3133.

### Compound 14

White solid, yield 21%. Rt (A) 20.07 min. IR: 3382, 2973, 2937, 2877, 1810, 1738, 1650, 1538, 1485, 1463, 1421, 1372, 1317, 1235, 1167, 1123, 1095, 1045, 1013, 985, 958, 904, 832, 805, 773, 727. $^{1}$H NMR ($CDCl_3$, δ ppm): 7.76 (m, 1H); 7.31 (m, 1H), 7.19 (m, 1H); 5.53 (s, 1H); 5.06 (d, 1H); 4.88 (m, 1H), 4.56 (m, 2H); 4.42 (s, 1H); 4.39 (s, 1H), 4.29 (m, 1H); 3.64 (m, 2H); 3.51 (d, 1H); 3.32–3.27 (m, 3H); 2.41–2.27 (m, 6H), 2.21–2.15 (m, 3H); 2.05 (s, 3H); 1.96–1.76 (m, 3H); 1.67–1.50 (m, 3H), 1.43 (s, 3H), 1.35–1.01 (m, 15H); 0.98–0.84 (m, 7H). $^{13}$C NMR ($CDCl_3$, δ ppm): 177.20, 170.09, 157.65, 153.31, 130.59, 125.45, 119.75, 114.60, 99.77, 94.76, 86.22, 84.72, 79.45, 76.55, 71.31, 67.63, 63.07, 61.33, 53.72, 49.37, 44.71, 41.38, 40.32, 35.05, 34.33, 30.54, 29.16, 26.77, 26.13, 21.96, 21.17, 20.95, 17.69, 13.69, 10.37, 5.10. MS (ESI) *m/z* cacl. for $C_{52}H_{83}BN_4O_{18}$ 1062.5795; found $(M + H)^+$ 1063.5843, $(M + H)^{2+}$ 532.2960.

### Compound 15

White solid, yield 17%. Rt (A) 20.36 min. IR: 3383, 2972, 2937, 2878, 1811, 1739, 1645, 1531, 1455, 1377, 1316, 1236, 1167, 1123, 1086, 1045, 1014, 985, 958, 904, 833,

805, 774, 731. $^{1}$H NMR ($CDCl_3$, δ ppm): 7.76–7.17 (m, 3H); 5.51 (s, 1H); 5.05 (d, 1H); 4.88 (m, 1H), 4.55 (m, 2H); 4.42 (s, 1H); 4.37 (s, 1H), 4.27 (m, 1H); 3.64 (m, 2H); 3.50 (d, 1H); 3.34–3.27 (m, 3H); 2.40–2.25 (m, 6H), 2.22–2.14 (m, 3H); 2.05 (s, 3H); 1.97–1.75 (m, 3H); 1.66–1.48 (m, 3H), 1.43 (s, 3H), 1.35–0.84 (23H). $^{13}$C NMR ($CDCl_3$, δ ppm): 177.17, 170.08, 157.65, 153.29, 130.59, 125.43, 119.76, 114.60, 99.75, 94.74, 86.20, 84.72, 79.42, 76.53, 71.20, 67.61, 63.04, 61.30, 53.72, 49.35, 44.70, 41.38, 40.30, 35.03, 34.31, 30.54, 29.16, 26.75, 26.13, 21.94, 21.17, 20.92, 17.69, 13.68, 10.35, 5.09. MS (ESI) *m/z* cacl. for $C_{52}H_{83}BN_4O_{18}$ 1062.5795; found $(M + H)^+$ 1063.5831, $(M + H)^{2+}$ 532.2862.

## Compound 16

White solid, yield 20%. Rt (A) 23.43 min. IR: 3383, 2971, 2933, 2874, 1810, 1740, 1651, 1600, 1537, 1453, 1377, 1353, 1236, 1165, 1124, 1106, 1087, 1043, 1012, 984, 957, 904, 932, 806, 774, 735. $^{1}$H NMR ($CDCl_3$, δ ppm): 7.35 (t, 1H); 7.18 (d, 1H), 7.12 (d, 1H); 6.60 (s. 1H); 5.67 (s, 1H); 5.08 (d, 1H); 5.03 (s, 1H); 4.88 (dd, 1H), 4.62 (d, 1H); 4.52 (d, 1H); 4.44 (s, 1H); 4.40 (s, 1H), 4.32 (m, 1H); 3.72 (m, 1H); 3.55 (d, 1H); 3.35 (s, 3H); 3.34–3.04 (m, 5H); 2.84–2.78 (m, 2H); 2.55 (t, 2H); 2.44–2.25 (m, 8H); 2.21 (s, 3H); 2.17 (d, 1H); 2.05 (s, 3H); 2.04–1.74 (m, 6H); 1.67–1.46 (m, 5H); 1.44 (s, 3H), 1.40–1.15 (m, 21H); 1.05 (d, 3H); 0.97–0.87 (m, 8H). $^{13}$C NMR ($CDCl_3$, δ ppm): 177.15, 173.92, 169.94, 156.89, 153.32, 145.43, 131.00, 126.92, 119.08, 114.57, 99.82, 94.86, 86.30, 86.27, 84.73, 83.29, 79.10, 76.22, 73.34, 71.60, 69.47, 67.76, 67.71, 63.14, 61.36, 53.74, 49.36, 43.07, 41.45, 40.50, 39.21, 37.17, 35.67, 35.14, 34.40, 31.67, 31.13, 29.61, 26.14, 22.06, 21.99, 21.19, 21.00, 17.83, 14.76, 13.72, 10.70, 10.38, 5.12. MS (ESI) *m/z* cacl. for $C_{55}H_{89}BN_4O_{18}$ 1104.6265; found $(M + H)^+$ 1105.6267, $(M + H)^{2+}$ 553.3174.

## Compound 17

White solid, yield 14%. Rt (A) 20.88 min. IR: 3375, 2973, 2938, 2877, 1810, 1739, 1645, 1538, 1455, 1428, 1377, 1315, 1236, 1167, 1124, 1106, 1086, 1045, 1013, 986, 958, 904, 833, 806, 773. $^{1}$H NMR ($CDCl_3$, δ ppm): 7.77 (m, 1H); 7.31 (m, 1H), 7.26 (m, 1H); 5.67 (s, 1H); 5.08 (d, 1H); 4.87 (dd, 1H), 4.61 (m, 1H); 4.43 (s, 1H); 4.40 (s, 1H), 4.33 (m, 1H); 3.72 (m, 1H); 3.54 (m, 1H); 3.34 (s, 3H); 3.31–3.27 (m, 2H); 3.04 (m, 1H); 2.84 (m, 1H); 2.45–2.40 (m, 1H); 2.35 (s, 3H); 2.21 (s, 2H), 2.05 (s, 3H); 2.01–1.47 (m, 7H); 1.43 (s, 3H), 1.31–1.13 (m, 13H); 1.05 (d, 3H); 0.98–0.87 (m, 6H). $^{13}$C NMR ($CDCl_3$, δ ppm): 177.53, 170.50, 157.62, 153.67, 145.68, 131.08, 125.81, 120.09, 100.17, 97.98, 95.17, 86.60, 85.07, 83.72, 79.57, 76.65, 73.74, 73.68, 71.68, 68.04, 63.42, 61.71, 58.66, 49.80, 45.10, 41.82, 40.68, 37.86, 35.44, 34.72, 31.12, 29.98, 27.15, 26.51, 22.34, 21.90, 21.58, 21.36, 18.13, 15.06, 14.08, 10.75, 5.48. MS (ESI) *m/z* cacl. for $C_{53}H_{85}BN_4O_{18}$ 1076.5952; found $(M + H)^+$ 1077.5975, $(M + H)^{2+}$ 539.3027.

## Compound 18

White solid, yield 10%. Rt (A) 21.13 min. IR: 3375, 2972, 2937, 2877, 1810, 1738, 1643, 1537, 1454, 1378, 1310, 1238, 1167, 1123, 1055, 1045, 1013, 986, 958, 904, 836, 805, 774, 731. $^{1}$H NMR ($CDCl_3$, δ ppm): 7.77–7.24 (m, 3H); 5.66 (s, 1H); 5.08 (d, 1H); 4.87 (m, 1H), 4.58 (m, 1H); 4.41 (m, 2H), 4.31 (m, 1H); 3.72 (m, 1H); 3.50 (m, 1H); 3.31–3.24 (m, 5H); 3.01 (m, 1H); 2.84 (m, 1H); 2.44–2.40 (m, 1H); 2.33 (s, 3H); 2.21 (m, 2H), 2.05 (s, 3H); 2.00–1.43 (s, 10H), 1.31–1.13 (m, 13H); 1.05 (d, 3H); 0.99–0.86 (m, 6H). $^{13}$C NMR ($CDCl_3$, δ ppm): 177.50, 168.45, 156.62, 151.42, 145.58, 130.76, 125.81, 118.17, 99.14, 98.04, 95.12, 85.98, 84.94, 83.57, 79.57, 76.55, 73.54, 72.98, 70.68, 68.07, 63.32, 61.71, 58.64, 48.76, 44.97, 41.78, 40.44, 37.76, 35.40, 34.67, 31.08, 29.90, 27.10, 25.47, 22.17, 21.77, 21.48, 21.36, 18.13, 14.87, 13.97, 10.72, 5.46. MS (ESI) *m/z* cacl. for $C_{53}H_{85}BN_4O_{18}$ 1076.5952; found $(M + H)^+$ 1077.6002, $(M + H)^{2+}$ 539.3038.

## Removing of acetyl group

A solution of the 2′-*O*-acetyl compound **13–18, 27, 28** in methanol was kept at 37 °C for 20 h. Subsequent concentration of the reaction solution in vacuum provided the desired product, which was purified by flash column chromatography eluting with 3:1 dichloromethane/ethanol to afford corresponding desired product.

## Compound 19

White solid, yield 77%. Rt (B) 17.22 min. IR: 3378, 2972, 2936, 2878, 1810, 1723, 1652, 1600, 1519, 1455, 1379, 1299, 1258, 1235, 1166, 1111, 1074, 1043, 1014, 985, 954, 903, 832, 804, 774. $^{1}$H NMR (DMSO-$d_6$, δ ppm): 7.17 (t, 1H); 6.97 (m, 2H), 6.73 (m, 1H), 6.41 (s, 1H); 6.08 (s, 1H); 4.87 (dd, 1H), 4.80 (m, 2H); 4.35 (d, 1H); 4.25 (m, 1H); 4.22 (s, 3H); 4.17 (m, 1H); 3.90 (dd, 1H); 3.54–3.47 (m, 2H); 3.45 (s, 3H); 3.37–3.11 (m, 7H); 3.11 (s, 3H); 3.08–2.60 (m, 9H); 2.35–2.20 (m, 5H); 2.16 (s, 3H); 2.05 (s, 3H); 1.97–1.85 (m, 3H); 1.82 (s, 3H); 1.76–1.30 (m, 6H); 1.28 (s, 3H); 1.14–0.70 (m, 20H). $^{13}$C NMR ($CDCl_3$, δ ppm): 177.36, 174.37, 170.09, 157.08, 154.95, 153.58, 145.63, 131.35, 119.41, 115.17, 100.20, 95.04, 86.60, 85.00, 79.44, 76.55, 71.93, 70.66, 67.92, 63.36, 61.68, 54.05, 49.68, 45.09, 41.79, 40.66, 40.39, 39.53, 35.35,

34.69, 31.14, 29.47, 27.14, 26.41, 18.14, 13.99, 10.68, 5.40. MS (ESI) *m/z* cacl. for $C_{52}H_{85}BN_4O_{17}$ 1048.6003; found $(M + H)^+$ 1049.5879, $(M + H)^{2+}$ 525.2961.

## Compound 20

White solid, yield 60%. Rt (A) 17.14 min. IR: 3392, 2972, 2934, 2877, 2850, 1810, 1731, 1649, 1536, 1462, 1380, 1314, 1259, 1234, 1167, 1121, 1074, 1044, 1014, 985, 958, 903, 835, 805, 773. $^1$H NMR ($CDCl_3$, δ ppm): 7.76 (m, 1H); 7.31 (m, 1H), 7.19 (m, 1H); 5.53 (s, 1H); 5.06 (d, 1H); 4.88 (m, 1H), 4.56 (m, 2H); 4.42 (s, 1H); 4.39 (s, 1H), 4.29 (m, 1H); 3.64 (m, 2H); 3.51 (d, 1H); 3.32–3.27 (m, 3H); 2.41–2.27 (m, 6H), 2.21–2.15 (m, 3H); 1.96–1.76 (m, 3H); 1.67–1.50 (m, 3H), 1.43 (s, 3H), 1.35–1.01 (m, 15H); 0.98–0.84 (m, 7H). $^{13}$C NMR ($CDCl_3$, δ ppm): 177.20, 170.09, 157.65, 153.31, 130.59, 119.75, 114.60, 99.77, 94.76, 86.22, 84.72, 79.45, 76.55, 71.31, 67.63, 63.07, 61.33, 53.72, 49.37, 44.71, 41.38, 40.32, 35.05, 34.33, 30.54, 29.16, 26.77, 26.13, 21.96, 21.17, 17.69, 13.69, 10.37, 5.10. MS (ESI) *m/z* cacl. for $C_{50}H_{81}BN_4O_{17}$ 1020.5690; found $(M + H)^+$ 1021.5618, $(M + H)^{2+}$ 511.2820.

## Compound 21

White solid, yield 78% Rt (A) 17.20 min. IR: 3388, 2973, 2936, 2876, 1811, 1728, 1643, 1537, 1462, 1381, 1315, 1253, 1238, 1168, 1122, 1074, 1044, 1014, 986, 958, 903, 834, 809, 772, 758. $^1$H NMR ($CDCl_3$, δ ppm): 7.76–7.17 (m, 3H); 5.50 (s, 1H); 5.04 (d, 1H); 4.88 (m, 1H), 4.55 (m, 2H); 4.42 (s, 1H); 4.37 (s, 1H), 4.27 (m, 1H); 3.64 (m, 2H); 3.50 (d, 1H); 3.34–3.27 (m, 3H); 2.40–2.25 (m, 6H), 2.22–2.14 (m, 3H); 1.97–1.75 (m, 3H); 1.66–1.48 (m, 3H), 1.43 (s, 3H), 1.35–0.84 (23H). $^{13}$C NMR ($CDCl_3$, δ ppm): 177.16, 170.07, 157.65, 153.29, 125.43, 119.76, 114.60, 99.75, 94.74, 86.20, 84.72, 79.42, 76.53, 71.20, 67.61, 63.04, 61.30, 53.72, 49.35, 44.70, 41.38, 40.30, 35.03, 34.31, 30.54, 29.16, 26.75, 26.13, 21.94, 20.92, 17.69, 13.68, 10.35, 5.09.

MS (ESI) *m/z* cacl. for $C_{50}H_{81}BN_4O_{17}$ 1020.5690; found $(M + H)^+$ 1021.5617, $(M + H)^{2+}$ 511.2814.

## Compound 22

White solid, yield 75%. Rt (B) 19.17 min. IR: 3382, 2972, 2935, 2877, 1810, 1721, 1653, 1600, 1539, 1454, 1379, 1300, 1258, 1236, 1166, 1111, 1073, 1045, 1014, 984, 958, 903, 833, 805, 774. $^1$H NMR ($CDCl_3$, δ ppm): 7.36 (t, 1H); 7.19 (m, 2H), 6.98 (m, 1H), 5.55 (m, 1H), 5.09 (m, 1H), 5.05 (s, 1H), 4.89 (m, 1H), 4.52 (t, 1H), 4.44 (m, 1H), 4.41 (s, 2H), 4.37 (m, 1H), 3.67 (m, 2H), 3.59 (d, 2H), 3.52 (s, 3H), 3.33 (s, 3H), 3.30–3.16 (m, 3H), 3.13 (m, 2H), 2.96 (t, 1H), 2.90–2.85 (m, 2H), 2.66 (m, 1H), 2.55 (t, 2H), 2.53–2.47 (m, 1H), 2.45–2.36 (m, 3H), 2.35 (s, 3H), 2.20 (s, 3H), 2.33 (s, 2H), 2.09–1.98 (m, 2H), 1.97–1.88 (m, 2H), 1.73–1.54 (m, 6H), 1.45 (s, 3H), 1.30 (s, 3H), 1.26–1.18 (m, 9H), 1.15 (s, 3H), 1.6 (d, 6H), 0.96–0.88 (m, 6H). $^{13}$C NMR ($CDCl_3$, δ ppm): 177.17, 176.75, 157.08, 153.36, 153.33, 146.68, 131.07, 128.52, 127.56, 127.04, 125.27, 119.11, 103.11, 95.34, 85.82, 85.11, 79.15, 77.99, 76.21, 73.40, 70.92, 68.44, 67.48, 64.92, 63.16, 61.12, 58.35, 49.53, 49.06, 45.28, 41.82, 40.28, 37.37, 35.20, 34.25, 33.87, 31.20, 26.79, 26.17, 25.55, 24.89, 21.96, 21.37, 21.01, 17.68, 14.89, 14.16, 10.36, 5.50. MS (ESI) *m/z* cacl. for $C_{53}H_{87}BN_4O_{17}$ 1062.6159; found $(M + H)^+$ 1063.6066, $(M + H)^{2+}$ 532.3061.

## Compound 23

White solid, yield 51%. Rt (A) 18.13 min. IR: 3405, 2973, 2940, 2879, 2836, 1810, 1714, 1644, 1537, 1463, 1381, 1315, 1259, 1231, 1169, 1124, 1074, 1045, 1014, 985, 957, 903, 840, 809, 773, 685. $^1$H NMR ($CDCl_3$, δ ppm): 7.77 (m, 1H); 7.31 (m, 1H), 7.26 (m, 1H); 5.67 (s, 1H); 5.08 (d, 1H); 4.87 (dd, 1H), 4.61 (m, 1H); 4.43 (s, 1H); 4.40 (s, 1H), 4.33 (m, 1H); 3.72 (m, 1H); 3.54 (m, 1H); 3.34 (s, 3H); 3.31–3.27 (m, 2H); 3.04 (m, 1H); 2.84 (m, 1H); 2.45–2.40 (m, 1H); 2.35 (s, 3H); 2.21 (s, 2H), 2.05 (s, 3H); 2.01–1.47 (m, 7H); 1.43 (s, 3H), 1.31–1.13 (m, 13H); 1.05 (d, 3H); 0.98–0.87 (m, 6H). $^{13}$C NMR ($CDCl_3$, δ ppm): 177.53, 170.50, 157.62, 153.67, 145.68, 131.08, 125.81, 120.09, 100.17, 97.98, 95.17, 86.60, 85.07, 83.72, 79.57, 76.65, 73.74, 73.68, 71.68, 68.04, 63.42, 61.71, 58.66, 49.80, 45.10, 41.82, 40.68, 37.86, 35.44, 34.72, 31.12, 29.98, 27.15, 26.51, 22.34, 21.90, 21.58, 21.36, 18.13, 15.06, 14.08, 10.75, 5.48. MS (ESI) *m/z* cacl. for $C_{51}H_{83}BN_4O_{17}$ 1034.5846, found $(M + H)^+$ 1035.5907, $(M + H)^{2+}$ 518.2989.

## Compound 24

White solid, yield 45%. Rt (A) 18.62 min. IR: 3359, 2971, 2935, 2876, 1809, 1713, 1641, 1538, 1454, 1380, 1354, 1311, 1259, 1231, 1167, 1120, 1084, 1043, 1013, 986, 956, 904, 838, 809, 774, 758. $^1$H NMR ($CDCl_3$, δ ppm): 7.75–7.24 (m, 3H); 5.65 (s, 1H); 5.07 (d, 1H); 4.85 (m, 1H), 4.57 (m, 1H); 4.42 (m, 2H), 4.30 (m, 1H); 3.70 (m, 1H); 3.50 (m, 1H); 3.32–3.24 (m, 5H); 3.00 (m, 1H); 2.84 (m, 1H); 2.42–2.40 (m, 1H); 2.21 (m, 2H), 2.03 (s, 3H); 2.00–1.43 (s, 10H), 1.33–1.13 (m, 13H); 1.05 (d, 3H); 0.99–0.86 (m, 6H). $^{13}$C NMR ($CDCl_3$, δ ppm): 177.44, 168.43, 156.62, 151.42, 145.58, 130.76, 125.81, 99.14, 98.04, 95.10, 85.98, 84.94, 83.57, 79.57, 76.55, 73.54, 73.01, 70.68, 68.06, 63.32, 61.71, 58.64, 48.76, 44.97, 41.78, 40.44, 37.76, 35.40, 34.67, 31.08, 29.90, 27.10,

25.47, 21.75, 21.48, 21.36, 18.13, 14.87, 13.97, 10.72, 5.45. MS (ESI) *m/z* cacl. for $C_{51}H_{83}BN_4O_{17}$ 1034.5846; found $(M + H)^+$ 1035.5853, $(M + H)^{2+}$ 518.2936.

## Compound 29

White solid, yield 10%. Rt (A) 17.20 min. IR: 3388, 2973, 2936, 2876, 1811, 1728, 1643, 1537, 1462, 1381, 1315, 1253, 1238, 1168, 1122, 1074, 1044, 1014, 986, 958, 903, 834, 809, 772, 758. $^1$H NMR (DMSO-$d_6$, δ ppm): 7.20–6.95 (m, 3H), 6.70 (m, 1H), 6.41 (s, 1H); 6.08 (s, 1H); 4.87 (dd, 1H), 4.80 (m, 2H); 4.35 (d, 1H); 4.25 (m, 1H); 4.22 (s, 3H); 4.17 (m, 1H); 3.90 (dd, 1H); 3.54–3.47 (m, 2H); 3.45 (s, 3H); 3.35–3.11 (m, 10H); 3.08–2.60 (m, 9H); 2.35–2.20 (m, 5H); 2.05 (s, 3H); 1.98–1.84 (m, 3H); 1.82 (s, 3H); 1.76–1.30 (m, 6H); 1.28 (s, 3H); 1.14–0.70 (m, 20H). $^{13}$C NMR ($CDCl_3$, δ ppm): 177.176, 175.34, 157.09, 154.95, 153.58, 145.63, 131.35, 119.41, 115.17, 100.20, 95.04, 86.60, 85.00, 79.44, 76.55, 71.93, 70.66, 67.92, 63.36, 61.68, 54.05, 49.68, 45.08, 41.78, 40.64, 40.17, 39.57, 35.37, 34.82, 31.14, 29.47, 27.14, 26.41, 18.14, 14.04, 10.45, 5.43. MS (ESI) *m/z* cacl. for $C_{50}H_{81}BN_4O_{17}$ 1020.5690; found $(M + H)^+$ 1021.5617, $(M + H)^{2+}$511.2814.

## Compound 30

White solid, yield 9%. Rt (A) 18.69 min. IR: 3355, 2974, 2935, 2879, 1925, 1810, 1713, 1659, 1455, 1416, 1379, 1324, 1273, 1165, 1120, 1088, 1045, 1012, 880, 804, 774 745, 701. $^1$H NMR ($CDCl_3$, δ ppm): 7.40–6.98 (m, 3H), 5.57 (m, 1H), 5.10 (m, 1H), 5.07 (s, 1H), 4.87 (m, 1H), 4.51 (t, 1H), 4.46 (m, 1H), 4.40 (s, 2H), 4.39 (m, 1H), 3.70 (m, 2H), 3.59–3.52 (m, 5H), 3.33 (s, 3H), 3.30–3.13 (m, 5H), 2.96 (t, 1H), 2.90–2.66 (m, 3H), 2.55 (m, 2H), 2.53–2.47 (m, 1H), 2.45–2.36 (m, 3H), 2.35 (s, 3H), 2.33 (s, 2H), 2.09–1.98 (m, 2H), 1.97–1.88 (m, 2H), 1.73–1.54 (m, 6H), 1.45 (s, 3H), 1.30 (s, 3H), 1.26–1.15 (s, 12H), 1.6 (d, 6H), 0.96–0.88 (m, 6H). $^{13}$C NMR ($CDCl_3$, δ ppm): 177.20, 176.57, 157.09, 153.44, 153.31, 146.71, 131.08, 128.44, 126.56, 125.04, 124.89, 119.10, 103.11, 95.34, 85.82, 85.11, 79.15, 77.99, 76.21, 73.40, 70.92, 68.44, 67.48, 64.92, 63.16, 61.12, 58.35, 49.53, 49.06, 45.28, 41.82, 40.28, 37.37, 35.20, 34.25, 33.87, 31.20, 26.79, 26.17, 25.55, 24.89, 22.04, 21.37, 21.12, 17.69, 14.92, 14.17, 10.36, 5.51. MS (ESI) *m/z* cacl. for $C_{52}H_{89}BN_4O_{16}$1036.6367; found $(M + H)^+$ 1037.6332, $(M + H)^{2+}$519.3206.

## In vitro antibacterial activity

MICs for all Gram-positive and Gram-negative bacterial strains were determined by standardized microdilution test using Mueller–Hinton broth (Acumedia, Baltimor) [20]. The bacterial inoculums contents of $5 \times 10^5$ CFU ml$^{-1}$ was incubated for 24 h at temperature 36 °C. Gram-positive (*St. aureus* ATCC 25923, *St. aureus* 10, *St. epidermidis* ATCC 12228, *S. pyogenes* ATCC 19615, *S. pneumoniae* ATCC 49619, *E. faecium* 568, *E. faecium* 569, *P. acnes* ATCC 6919) and Gram-negative strains (*E.coli* ECM 1888, *E. coli* leuS ANA395, *P. multocida* ATCC 11039, *M. haemolytica* ATCC 33396, *H. somni* ATCC 700025) were used in the experiments. All the strains were from American Type Culture Collection (ATCC) or clinical isolates.

## Dual-fluorescent-protein reporter assay in liquid medium

The experiment was carried out as described [19]. The overnight culture of ΔtolC *E. coli* cells, transformed by pRFPCER-TrpL2A (translation reporter), was diluted to 0.05 to 0.1 OD (590 nm) units with fresh LB medium supplied with ampicillin 100 μg ml$^{-1}$ and plated on LB agar medium (ampicillin 100 μg ml$^{-1}$). After drying of the plate, 2 μl of the 10 mg ml$^{-1}$ tested solutions was added to spotted. As a negative control of the translation inhibition, we used levofloxacin (3 mg ml$^{-1}$), and as a positive control of the translation inhibition we used erythromycin (3 μg ml$^{-1}$). Following overnight incubation at 37 °C, the Petri dishes were illuminated at UV (254 nm) and documented by a digital camera.

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## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

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