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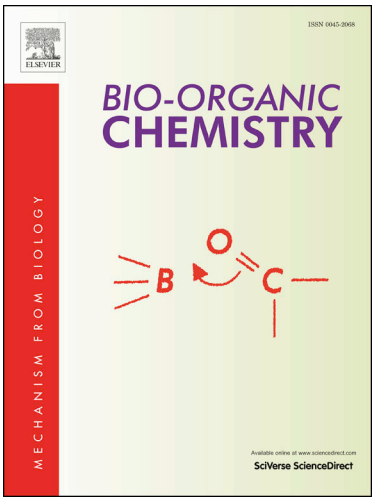

Synthesis and antimicrobial activity of (−)-cleistenolide and analogues

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Graphical Abstract

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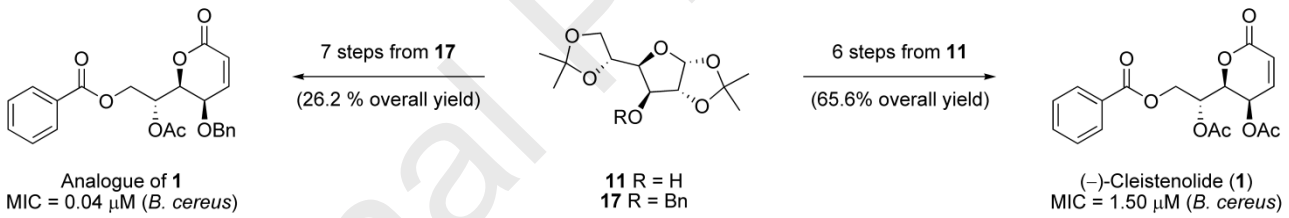

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Abstract

Using the "chiral pool" approach, two modified total syntheses of the biologically active δ-lactone cleistenolide (**1**) have been achieved starting from D-glucose. These approaches also enabled the preparation of novel analogues and derivatives of natural product **1**. The applied strategy for the synthesis of **1** involves: the initial degradation of the chiral precursor for a single C-atom, C_2-fragment chain extension using Z-selective Wittig reaction, and the final δ-lactonization. All tested cleistenolide analogues displayed antimicrobial activity against a panel of nine microbial strains, most of them superseding the activity of cleistenolide itself, and, in some cases, coming close in value to the observed minimal inhibitory concentrations of chloramphenicol. Increased lipophilicity of the derivatives and the non-sterically congested conjugated lactone moiety were a prerequisite for analogues with high inhibitory activity against *S. aureus* and, in general, Gram-positive bacteria.

Keywords: cleistenolide; synthesis of analogues; pyranones; antimicrobial lactones; hierarchical clustering

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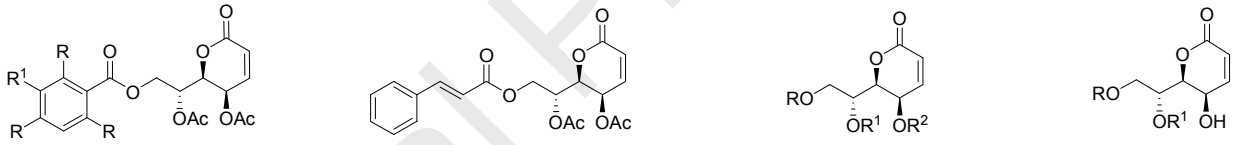
1. Introduction

(−)-Cleistenolide (**1**, Figure 1), is a naturally occurring α,β-unsaturated δ-lactone that was isolated from *Cleistochlamys kirkii* Oliver, and exhibited in vitro antibacterial activity against *Staphylococcus aureus* and *Bacillus anthracis* and antifungal activity against *Candida albicans* [1]. A recent study additionally revealed that (−)-cleistenolide (**1**) exhibits antibacterial activity against some Gram-positive strains [2]. Ten total syntheses of the natural product **1** have been completed so far [3], using different methods such as ring closing metathesis [3–8], *Z*-selective olefination [9–11] or Yamaguchi lactonization [12] as the key steps. The most efficient route (49% yield over eight steps) is achieved by Du, Linhardt et al. [12] starting from D-arabinose. The second synthesis by efficiency was realized starting from D-glucose, in six steps, and in an overall yield of 42.5% [9].

Despite the existence of these numerous synthetic pathways to **1**, there is still a demand to develop alternative synthetic procedures, especially those that would enable the preparation of novel cleistenolide analogues.

Herein, we present two alternative approaches to the total syntheses of (−)-cleistenolide (**1**), and its new analogues **3**–**10**, as well as their preliminary antimicrobial screening.



1 R = R^1 = H (Cleistenolide)
2 R = H, R^1 = OMe
3 R = Cl, R^1 = H

4

5 R = R^1 = R^2 = Bn
6 R = Bz, R^1 = Ac, R^2 = Bn
7 R = R^1 = R^2 = Bz
8 R = R^1 = R^2 = 3-MeOBz

9 R = R^1 = H
10 R = Bz, R^1 = Ac

Fig. 1. Chemical structures of (−)-cleistenolide (**1**) and the corresponding analogues (**2**–**10**).

Compounds **3** and **2** are direct isosteres of the lead **1**, while the analogue **4** represents a new hybrid of cinnamic acid and the natural product **1**. Cinnamate hybrids are a unique family of compounds with multiple biological activities [14], including antimicrobial activity [15]. Finally, we have designed four highly hydrophobic (**5**–**8**) and two hydrophilic derivatives (**9** and **10**) having an α,β-unsaturated δ-lactone scaffold. The rationale for obtaining this type of compound is to evaluate the effect of their lipophilic/hydrophilic properties on antimicrobial activity.

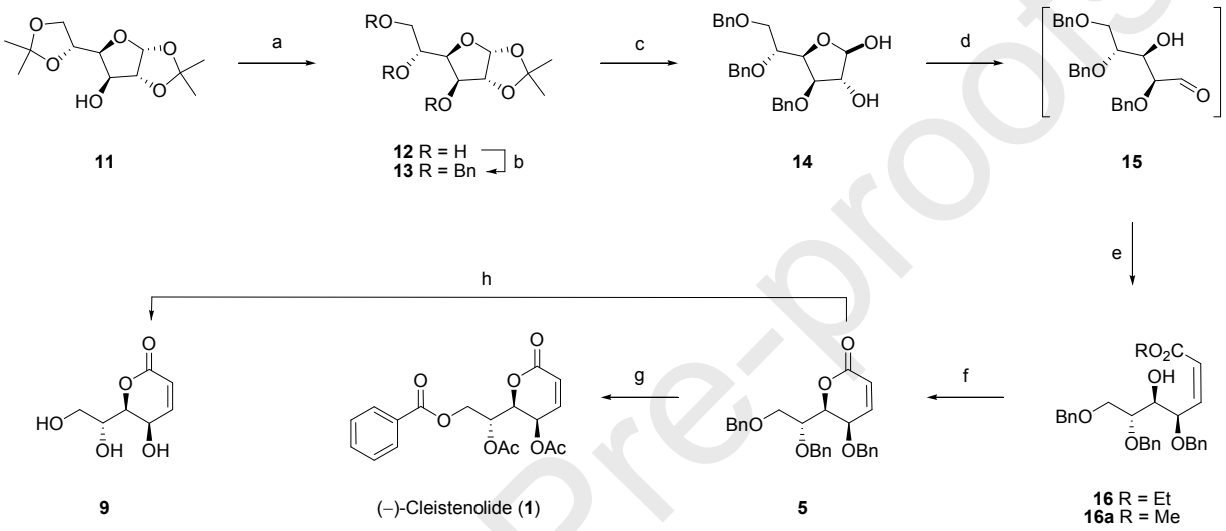
Part of this work was recently published in the form of a preliminary communication, describing that the natural product **1** and some of its analogues strongly inhibit the growth of selected tumour cells [13]. Considering the problem of bacteraemia and invasive candidiasis in

cancer patients, an important task of this work was to prepare and test the antimicrobial activity of **1** and related compounds for a possible use in multifunctional therapy [16].

2. Results and discussion

2.1. Chemistry

The synthesis of **1** and **9** is summarized in Scheme 1. The sequence started from commercially available diacetone D-glucose **11**.



Scheme 1. Reagents and conditions: (a) 60% aq AcOH, 24 h, rt; (b) NaH, DMF, 0 °C (1 h) → rt (2 h), 90% from **11**; (c) 50% aq TFA, 18 h, rt, 83%; (d) H_5IO_6, H_2O, EtOAc, 2.5 h, rt; (e) Ph_3P=CH-CO_2Et, MeOH, 20 h, rt, 86% from **14**; (f) TsOH, CH_2Cl_2, 12 h, rt, 92%; (g) BzBr, $FeCl_3$, rt, 2 h, then AcBr, rt, 20 h, 74%; (h) $FeCl_3$, CH_2Cl_2, 20.5 h, rt, 92%.

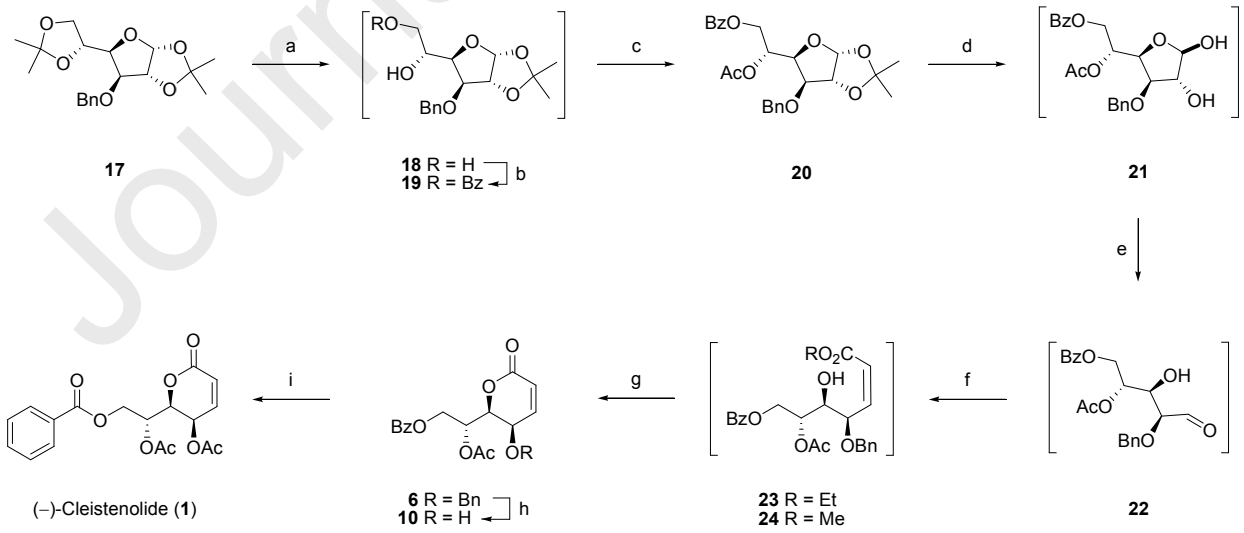
Hydrolytic removal of the terminal isopropylidene protection produced monoacetonide **12** which was not purified, but was subsequently treated with benzyl bromide in dry DMF, in the presence of sodium hydride, to give the known [17,18] tri-*O*-benzyl derivative **13** in 90% overall yield (from two steps). Hydrolytic removal of the isopropylidene protecting group in **13** gave the corresponding lactol **14** as only reaction product. Although lactol **14** is most likely a mixture of α- and β-anomer, their mutual ratio cannot be determined due to the complexity of both ^{1}H and ^{13}C NMR spectra. The purity of product **14** was confirmed by HRMS data [*m/z* 451.2112 (M^++H), calcd for $C_{24}H_{27}O_8$: 451.2115]. Oxidative cleavage of **14** with periodic acid, followed by *Z*-selective Wittig olefination [19,20] of the resulting aldehyde **15** with stabilized C_2-ylide (Ph_3P=$CHCO_2Et$), afforded the unsaturated ester **16** (72% from the last three steps). The unsaturated ester **16** cyclised to **5** after treatment with toluene-4-sulfonic acid in anhydrous dichloromethane. The desired lactone **5** was thus obtained in a yield of 92%. Treatment of **5** with BzBr/$FeCl_3$ reagent system resulted in the selective introduction of the benzoyl group into the primary position. The subsequent *in situ*

acetylating of the resulting diol (not shown in the Scheme 1) yielded the natural product **1** in 74% yield. This modified synthesis of **1** was accomplished in seven synthetic steps, in a total yield of 44%. In terms of yield, this synthesis is slightly less efficient than the synthesis reported by Du, Linhardt et all. [11] (49% from eight steps). However, our synthesis is for one step shorter. In order to optimize the initial stages of the synthesis, sodium periodate was used for diol cleavage, the Wittig reaction was performed using $Ph_3P{=}CH\text{-}CO_2Me$, and the initial five-step sequence was carried out without isolation or purification of intermediates **12**, **13**, **14** and **15**. This provided the key intermediate **16a** in a total yield of 75% from five synthetic steps.

This approach to the synthesis of (−)-cleistenolide (**1**) is somewhat similar to the Yadav's route that proceeded in six steps from D-glucose diacetonide, in 42.5% overall yield [9]. Our approach (Scheme 1) initially provided natural product **1** in almost equal total yield (44% from the seven synthetic steps). However, the optimization of some key steps, as mentioned above increased the yield of target 1 to 65.6% (from six steps).

Finally, to obtain the triol **9**, which is a possible divergent intermediate for the synthesis of various new (−)-cleistenolide analogues and derivatives, $FeCl_3$-mediated global deprotection of benzyl groups in **5** was performed to obtain the desired triol **9** in 92% yield.

An alternative synthesis of cleistenolide (**1**) is shown in Scheme 2. The 3-*O*-benzyl-1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose (**17**), which is readily available from D-glucose [21], was used as a convenient starting compound in this approach.



Scheme 2. Reagents and conditions: (a) 60% aq AcOH, rt, 24 h; (b) BzCl, DMAP, CH_2Cl_2, rt, 1 h; (c) AcCl, DMAP, rt, 1 h, 69% from **17**; (d) 90% aq TFA, CH_2Cl_2, 0 °C (0.5 h) → rt (2 h); (e) H_5IO_6, EtOAc, H_2O, 0 °C (0.5 h) → rt (3.5 h); (f) $Ph_3P{=}CH\text{-}CO_2Et$, MeOH, rt, 20 h; (g) TsOH, CH_2Cl_2, rt, 20 h, 38% from **20**; (h) $TiCl_4$, CH_2Cl_2, rt, 24 h, 70%; (i) Ac_2O, TsOH, rt, 4 h, 95%.

Selective hydrolytic removal of the exocyclic isopropylidene protection in **17** gave the corresponding diol **18**, which was not purified but allowed to react in crude form with BzCl in anhydrous CH_2Cl_2, in the presence of DMAP. Only the primary OH group is benzoylated under these reaction conditions (~1 eq of BzCl, rt, 1 h). Subsequent *in situ* acetylating of the resulting secondary alcohol **19** gave the known [22] fully protected derivative **20** in a total yield of 69% (from 3 steps). Compound **20** was further treated with 9:1 aqueous trifluoroacetic acid in CH_2Cl_2, at 0 °C for 0.5 h, then at room temperature for 2 h, to give the corresponding lactol **21**. The product is a mixture of the corresponding α- and β-anomers (β/α = 2:1) as determined by the integration of anomeric proton signals in the ^{1}H NMR spectrum [δ 5.23 (s, 1 H, H-1β), 5.54 (d, 0.5 H, $J_{1,2}$ = 4.0 Hz, H-1α)]. Oxidative cleavage of **21** with periodic acid in an ethyl acetate and water gave the aldehyde **22**. Due to its instability the aldehyde **22** was subjected directly to a C_2 homologation using *Z*-selective olefination conditions [19] to afford the corresponding *Z*-enoates as a 1:1 mixture of ethyl (**23**) and methyl ester (**24**). The methyl ester **24** is obviously the product of trans-esterification of **23** with methanol. The similar reaction was not observed in the Wittig reaction of **15** under the same conditions (Scheme 1). The mixture of esters was not separated, but was further converted to the pyranone **6**, after treatment with toluene-4-sulfonic acid in anhydrous dichloromethane. Lactone **6** was obtained in 38% overall yield (four steps). Deprotection of the benzyl group in **6** was achieved with titanium(IV) chloride in anhydrous dichloromethane, to afford alcohol **10** in 70% yield. Acetylation of **10** (Ac_2O, TsOH, rt, 4 h) gave the natural product **1** in 95% yield. Although the overall yield of this synthetic pathway is lower (17.4% from 9 steps) than the previous one, it has an important advantage because it provides access to a divergent intermediate (**10**) for the synthesis of various novel C-4 substituted analogues.

The melting point and spectral data of thus obtained synthetic sample **1** were in excellent agreement with data reported in the literature [3–13]. ^{1}H and ^{13}C NMR spectra (see the Supplementary material) confirmed the purity of the synthesized natural product. However, the value of optical rotation was different. We observed the values of $[\alpha]_D = -212.2$ (*c* 0.5, $CHCl_3$), and $[\alpha]_D = -212.4$ (*c* 0.5, CH_2Cl_2). Lit [1] recorded for the isolated natural product: $[\alpha]_D = -63.5$ (*c* 0.7, $CHCl_3$); the closest literature [8] value for the synthetic sample: $[\alpha]_D = -164.6$ (*c* 0.5, CH_2Cl_2). At the moment, we have no explanation for this discrepancies.

The conversion of triol **9** to cleistenolide analogues **2**–**4**, and related 5,6-dihydro-2*H*-pyran-2-one derivatives **7** and **8** is shown in Table 1.

Table 1. Preparation of (−)-cleistenolide analogues **2**–**4**, **7** and **8**.

Entry	Reagents and conditions	Ar	R	Product (yield)
1	(a) 3-methoxybenzoyl chloride, Py/CH_2Cl_2, rt, 4 h; (b) Ac_2O, rt, 20 h	3-methoxyphenyl	Ac	**2** (67%)
2	(a) 2,4,6-trichlorobenzoyl chloride, Py/CH_2Cl_2, rt, 4 h; (b) Ac_2O, rt, 20 h	2,4,6-trichlorophenyl	Ac	**3** (85%)
3	(a) cinnamoyl chloride, Py/CH_2Cl_2, rt, 2 h; (b) Ac_2O, rt, 18 h	styryl	Ac	**4** (47%)
4	benzoyl chloride, Py/CH_2Cl_2, rt, 3.5 h	phenyl	Bz	**7** (86%)
5	3-methoxybenzoyl chloride, Py/CH_2Cl_2, rt, 3.5 h	3-methoxyphenyl	3-methoxy-Bz	**8** (88%)

The first three analogues **2**–**4** were prepared through a two-step sequence consisting of the initial selective arylation of **9** with one molar equivalent of ArCOCl, followed by acetylating of the intermediately formed diol (entries 1–3). The cleistenolide isosteres **2** and **3** were obtained as the only reaction products in good yields (entries 1 and 2), while the cinnamic acid hybrid (**4**) was obtained in a slightly lower yield (entry 3). Treatment of **9** with six molar equivalents of benzoyl chloride, or 3-methoxybenzoyl chloride gave excellent yields of the corresponding ester derivatives **7** (entry 4), or **8** (entry 5).

All these analogues (Table 1), along with the lead **1** and 5,6-dihydro-2*H*-pyran-2-one derivatives (**5**, **6, 9** and **10**), used as intermediates in the synthetic pathways described (Schemes 1 and 2), were evaluated for their antimicrobial activity.

2.2. Antimicrobial activities

Cleistenolide and the mentioned analogues were evaluated for their antimicrobial activity against a panel of ATCC microbial strains, four Gram-positive and four Gram-negative bacteria, and one yeast. The tested compounds were shown to possess inhibitory action (MIC) in the range 0.04–6.00 μM/ml (Table 2) and exhibited a generally wide spectrum of activity by affecting the

Table 2. Antimicrobial activity (MIC in μmol/ml) of cleistenolide and its analogues.

Strain/compound	***1***	***2***	***3***	***4***	***5***	***6***	***7***	***9***	***8***	***10***	***CHL****	***NYS****
Gram-positive bacteria												
S. aureus	0.75	1.50	0.37	0.37	1.50	0.37	0.18	6.00	0.37	0.75	19.4	NT
S. epidermidis	0.75	0.75	3.00	0.75	0.75	1.50	1.50	>6.00	3.00	0.75	4.82	NT
K. rhizophila	0.37	0.75	0.18	0.09	6.00	0.09	0.09	6.00	0.75	0.18	2.41	NT
B. cereus	1.50	1.50	0.18	0.75	6.00	0.04	3.00	6.00	6.00	0.75	9.65	NT
Gram-negative bacteria												
E. coli	6.00	6.00	6.00	6.00	6.00	1.50	3.00	6.00	>6.00	3.00	4.82	NT
P. aeruginosa	6.00	6.00	6.00	3.00	3.00	6.00	6.00	6.00	3.00	6.00	38.68	NT
A. baumannii	3.00	3.00	3.00	1.50	6.00	1.50	6.00	6.00	3.00	1.50	38.68	NT
S. enteritidis	6.00	6.00	6.00	6.00	6.00	3.00	6.00	6.00	6.00	6.00	9.65	NT
Yeast												
C. albicans	3.00	1.50	3.00	1.50	6.00	0.37	6.00	6.00	>6.00	6.00	NT	2.52

CHL – chloramphenicol; NYS – nystatin; * – results given in nM/ml, NT – not tested.

growth of all tested strains. The only exceptions were **8** and **9** which failed to be active at the tested concentration range against two and one strains, respectively.

The highest activity was observed in the case of compound **6** against Gram-positive *B. cereus*, where the growth inhibition was achieved at the concentration of 40 nM/ml, which was only ca. 4 times lower compared to chloramphenicol. Considering the overall susceptibility of the strains, the lowest average MIC of 1.2 μM/ml was determined for *S. aureus*. Among the tested compounds, **4** and **6** exhibited the highest antimicrobial effect with average MIC values of 2.21 and 1.59 μM/ml, respectively. On the other hand, the lowest antimicrobial action was noted in the case of compounds **5** and **9**, which had average MIC values of 4.58 and 6.00 μM/ml, respectively. Among the tested compounds, it appears that the Gram-positive strains showed a significantly higher susceptibility to the action of all tested compounds. On the other hand, the yeast, *C. albicans*, seemed to be affected in a similar way to that observed for Gram-negative strains. Among the tested strains, the Gram-negative strains *S. enteritidis* and *P. aeruginosa* demonstrated the highest resistance.

To further obtain insights into the antimicrobial nature of cleistenolide and its congeners, a hierarchical clustering analysis of the activity data was undertaken. Minimal inhibitory concentrations were utilized in the statistical treatment and the results are presented in Figure 2 as the heatmap and two dendrograms.

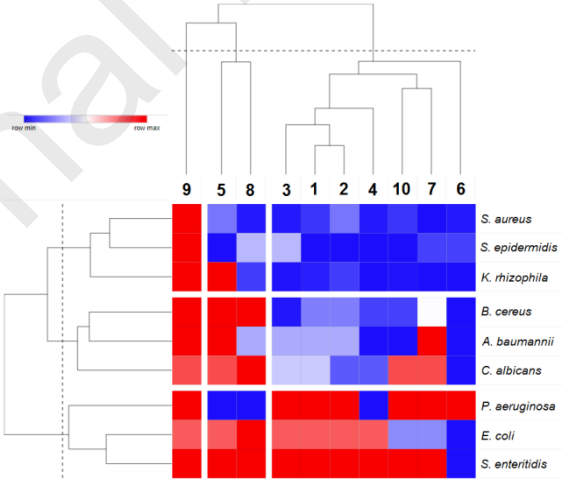


Fig. 2. Heatmap representing color-coded MIC values of the tested cleistenolide and its analogues with row and column dendrograms obtained by a hierarchical clustering analysis (Euclidian distance and average linkage clustering method.

Clearly visible is the mentioned higher activity of almost all cleistenolide analogues toward Gram-positive bacteria (a cluster of three bacteria *S. aureus*, *S. epidermidis*, and *K. rhizophila*), as well as a lower susceptibility of the majority of Gram-negative strains (the cluster composed of *P.*

aeruginosa, *E. coli*, and *S. enteritidis*). Anticandidal activity was intermediate, as that observed for one Gram-positive (*B. cereus*) and Gram-negative (*A. baumannii*). This pronounced, alas unquantified [1], activity against *S. aureus* was noted initially when cleistenolide was originally isolated. The seminal work [1] states, again without MIC values, that cleistenolide in addition to antistaphylococcal activity was active against *B. anthracis* and *C. albicans*.

Based on the clustering of the tested derivatives, it is evident data the most hydrophilic tested compound (**9**) was the least active one, while still preserving some unselective activity in the highest tested concentration. The sterically congested (second cluster) per-*O*-benzyl ether derivative (**5**) and the electron-rich trimethoxybenzoate (**8**) appear to have a somewhat decreased antimicrobial effect compared to the mostly acetyl derivatives (the remaining compounds forming the third cluster). It follows that the likely pharmacophore is the Michael acceptor, α,β-unsaturated lactone, as all of the derivatives showed a certain degree of activity; however, two factors influence the expression of this activity: lipophilicity and steric hindrance, the first being positive, and the second one negatively influencing the electrophilicity of the conjugated system. The lack of lipopolysaccharides in Gram-positive bacteria, while these are a major component of the outer membrane of Gram-negative bacteria, contributing greatly to the structural integrity of the bacteria, appears to correlate well with the observed higher susceptibilities of Gram-positive bacteria; it suggests the cell membrane as a possible cellular target of cleistenolide and hence the increased lipophilicity as a structural requirement of higher active analogues. It seems that the activities could be fine-tuned by altering the identities of the esterifying aromatic acids furthest to the pharmacophore and by minding the steric requirements of the remaining, preferentially lipophilic, more proximal *O*-substituents.

3. Conclusions

Two independent synthetic sequences have been developed for the total synthesis of the natural biologically active lactone cleistenolide (**1**), starting from commercially available D-glucose derivatives. The first route (Scheme 1) represents a modified procedure recently published as a preliminary communication [4]. After optimizing the reaction conditions of several key synthetic steps, the target **1** was obtained in an overall yield of 43.7% from seven steps. Obviously, the modified procedure is significantly more efficient than that described in the preliminary communication [4] which gave the target **1** in an overall yield of 33% (from seven steps). However, after additional optimizations of the initial stages of the synthesis, the natural product **1** was obtained in a total yield of 65.6% over 6 steps. This is the most efficient total synthesis of (−)-

cleistenolide so far. The second synthetic pathway (Scheme 2) gave the natural product **1** in a lower overall yield (17.4% from 9 steps). Nevertheless, this approach has the important advantage of providing access to an important intermediate (compound **10**), suitable for the preparation of a variety of novel C-4 substituted analogues. By combining both approaches mentioned above, several novel analogues and derivatives of **1** were synthesized and their antimicrobial activity was evaluated. The structural variations in the tested cleistenolide derivatives all resulted in compounds that possess a wide range of antimicrobial activities, and that display, in some cases, MIC values close in to that of commercial antibiotic chloramphenicol. Increased lipophilicity of the derivatives and the non-sterically congested conjugated lactone moiety were a prerequisite for analogues with high inhibitory activity against *S. aureus* and, in general, Gram-positive bacteria.

4. Experimental section

4.1.1. General experimental procedures

For general experimental procedures and for the preparation of initial intermediate **17**, see the Supplementary data.

*4.1.2. 3,5,6-Tri-O-benzyl-1,2-O-isopropylidene-α-D-glucofuranose (**13**)*

A solution of **11** (2.45 g, 9.41 mmol) in 60% aq AcOH was stirred at room temperature for 24 h and then evaporated by co-distillation with toluene. The remaining crude **12** was dried in high vacuum over night. To a cooled (0 °C) and stirred solution of crude **12** in anhydrous DMF (70 mL) was slowly added 95% NaH (2.0 g, 83.33 mmol) in portions during 0.5 h. When the evolution of hydrogen was ceased, BnBr (2.0 mL, 16.82 mmol) was added and the resulting mixture was stirred at 0 °C for 0.5 h. The cooling bath was removed and the stirring was continued at room temperature for 2 h. The excess of NaH was destroyed by treatment with absolute MeOH (10 mL) and the volatiles were evaporated. The residue was distributed between CH_2Cl_2 (40 mL) and H_2O (300 mL), and extracted successively with CH_2Cl_2 (3 × 40 mL) and EtOAc (30 mL). Organic phases were combined, dried and evaporated. The remaining crude mixture was purified on a column of flash silica (3:1 light petroleum/Et_2O) to give pure **13** (4.179 g, 90%) as a colourless glassy solid, $[\alpha]_D^{23}$ = −45.2 (c 1.0, $CHCl_3$), lit. [17] $[\alpha]_D$ = −33.0 (*c* 9.3, $CHCl_3$), lit. [18] $[\alpha]_D$ = −61.8 (c 0.3, $CHCl_3$), R_f = 0.42 (7:3 light petroleum/Et_2O). IR (film): ν_{max} 1605, 1586, 1078, 1027. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.39 and 1.58 (2 × s, 3 H each, Me_2C), 3.79 (dd, 1 H, $J_{5,6a}$ = 5.8, $J_{6a,6b}$ = 10.6 Hz, H-6a), 4.01 (dd, 1 H, $J_{5,6b}$ = 1.7, $J_{6a,6b}$=10.6 Hz, H-6b), 4.17 (ddd, 1 H, $J_{5,6b}$ = 1.7, $J_{5,6a}$ = 5.8, $J_{4,5}$ = 9.3 Hz, H-5), 4.22 (d, 1 H, $J_{3,4}$ = 2.9 Hz, H-3), 4.42 (dd, 1 H, $J_{3,4}$ = 2.9, $J_{4,5}$ = 9.3 Hz, H-4), 4.54–4.94 (m, 7

H, H-2 and 3 × C*H*$_2$Ph), 6.00 (d, 1 H, $J_{1,2}$ = 3.6 Hz, H-1), 7.28–7.56 (m, 15 H, 3 × Ph). HRMS (ESI): *m/z* 513.2239 (M^++Na), calcd for $C_{30}H_{34}NaO_6$: 513.2248.

*4.1.3. Ethyl (2Z)-4,6,7-tri-O-benzyl-2,3-dideoxy-D-arabino-hept-2-enoate (**16**)*

A solution of **13** (2.16 g, 0.28 mmol) in 50% aq TFA (30 mL) was stirred at room temperature for 18 h. The solution was evaporated by co-distillation with toluene to remove the traces of acid and water. The residue was purified by flash column chromatography (3:1 Et_2O/light petroleum) to afford pure **14** as a colourless glassy solid, R_f = 0.45 (4:1 Et_2O/light petroleum). IR (film): ν_{max} 3405, 1605, 1586, 1061, 1028 cm^{-1}. ^{1}H NMR (250 MHz, $CDCl_3$): All signals are heavily overlapped. ^{13}C NMR (62.5 MHz, $CDCl_3$): δ 70.4, 70.8, 71.8, 72.3, 72.5, 72.6, 73.4, 73.5, 73.8, 73.9, 76.1, 76.7, 77.7, 80.0, 82.2, 83.4, 96.9, 103.4, 127.4, 127.5, 127.6, 127.65, 128.1, 128.2, 128.26, 128.3, 128.6, 137.8, 138.2, 138.5. HRMS (ESI): *m/z* 451.2112 (M^++H), calcd for $C_{24}H_{27}O_8$: 451.2115. To a stirred solution of purified **14** (1.6 g, 3.55 mmol) in EtOAc (20 mL) were successively added H_2O (20 mL) and H_5IO_6 (1.57 g, 6.89 mmol) and the resulting solution was stirred at room temperature for 2.5 h. The mixture was poured to 10% aq NaCl (50 mL) and extracted with EtOAc (3 × 30 mL). The combined organic layers were dried and evaporated and the residue was dried in high vacuum for 12 h. The resulting crude **15** was dissolved in MeOH (125 mL) and treated with 2-(triphenylphosphoranylidene)-acetic acid ethyl ester (3.6 g, 10.33 mmol). The mixture was stirred at room temperature for 20 h and then evaporated. The residue was purified on a column of flash silica (3:2 light petroleum/Et_2O) to give pure **16** (1.5 g, 72% from **13**) as a colourless glassy solid, $[\alpha]_D^{23}$ = −10.4 (*c* 1.0, $CHCl_3$), R_f = 0.33 (3:2 light petroleum/Et_2O). IR (film): ν_{max} 3476, 1717, 1650, 1605, 1586, 1028. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.23 (t, 3 H, *J* = 7.2 Hz, CH_2CH_3), 2.51 (bs, 1 H, OH), 4.19 (q, 2 H, CH_2CH_3), 3.70–3.93 (m, 4 H, H-7a, H-7b, H-6 and H-5), 4.40, 4.50, 4.59, 4.74 (4 × d, 4 H, J_{gem} = 11.6 Hz, 2 × C*H*$_2$Ph), 4.59 (s, 2 H, C*H*$_2$Ph at C-7), 5.45 (ddd, $J_{3,4}$ = 8.8, $J_{2,4}$ = 0.8, $J_{4,5}$ = 2.5 Hz, H-4), 6.02 (d, 1 H, $J_{2,3}$ = 11.8 Hz, H-2), 6.40 (dd, 1 H, $J_{2,3}$ = 11.8, $J_{3,4}$ = 9.0 Hz, H-3), 7.24–7.39 (m, 15 H, 3 × Ph). The sample is contaminated with 4% of *E*-isomer. ^{13}C NMR (100 MHz, $CDCl_3$): 14.1 ($CO_2CH_2CH_3$), 60.3 ($CO_2CH_2CH_3$), 70.7 (C-7), 71.2, 72.3, 73.4 (3 × CH_2Ph), 73.6 (C-4), 74.4 (C-5), 77.8 (C-6) 122.8 (C-2), 127.4, 127.5, 127.59, 127.6, 127.7, 127.8, 127.83, 127.9, 128.0, 128.2, 128.26, 128.3, 128.33, 128.4, 137.9, 138.2, 138.5 (Ph), 146.9 (C-3), 165.6 ($CO_2CH_2CH_3$). HRMS (ESI): *m/z* 513.2240 (M^++Na), calcd for $C_{30}H_{34}NaO_6$: 513.2253.

*4.1.4. Methyl (2Z)-4,6,7-tri-O-benzyl-2,3-dideoxy-D-arabino-hept-2-enoate (**16a**)*

A solution of **11** (3 g, 11.53 mmol) in 60% aq AcOH (45 mL) was stirred at room temperature for 24 h, then evaporated by co-distillation with toluene to remove the traces of acid and water. To a stirred and cooled (0 °C) solution of **12** in anhydrous DMF (75 mL) was added NaH (1.66 g, 69.16 mmol) and after cessation of intensive H_2 evolution, BnBr (4.8 mL, 40.34 mmol) was slowly added over 10 minutes. The mixture was stirred at 0 °C for 0.5 h, the cooling bath was then removed and stirring at room temperature was continued for additional 2 h. Absolute MeOH (12 mL) was slowly added to the reaction solution. After cessation of intensive evolution of hydrogen, the mixture was evaporated. The residue was partitioned between CH_2Cl_2 (40 mL) and H_2O (250 mL), the organic layer was separated and extracted with CH_2Cl_2 (3 × 40 mL). The organic phases were combined, washed with 10% aq NaCl (3 × 150 mL), dried and evaporated. The residue (crude **13**) was treated with 50% aq TFA (60 mL) while vigorous stirring at room temperature for 18 h. The solution was evaporated with toluene to completely remove the acid and water and the resulting residue was dried in high vacuum overnight. To a solution of crude **13** in absolute MeOH (500 mL), was added $NaIO_4$ (3.68 g, 17.29 mmol) and the mixture was stirred at room temperature for 2 h. The mixture was cooled to 0 °C and treated with $Ph_3P{=}CHCO_2Me$ (7.5 g, 22.46 mmol). After 1 h, the cooling vessel was removed and the reaction continued at room temperature for 3 h. A new portion of $Ph_3P{=}CHCO_2Me$ (4.05 g, 12.12 mmol) was then added and stirring continued for additional 20 h. The reaction mixture was evaporated and the residue was purified twice by means of flash column chromatography (1:1 light petroleum/Et_2O). Pure product **16a** (4.1 g, 75% from **11**) was obtained in the form of colourless syrup, $[\alpha]_D = -19.1$ (*c* 1.0, $CHCl_3$). $R_f = 0.32$ (3:2 light petroleum/Et_2O). The IR, NMR, and HRMS of the thus obtained **16a** were fully consistent with the values previously reported by us [4].

*4.1.5. 4,6,7-Tri-O-benzyl-2,3-dideoxy-D-arabino-hept-2-eno-1,5-lactone (**5**)*

Procedure A. A solution of **16** (1.55 g, 3.16 mmol) and $TsOH{\cdot}H_2O$ (0.015 g, 0.08 mmol) in CH_2Cl_2 (120 mL) stirred at room temperature for 12 h and then evaporated. The residue was purified on a column of flash silica (1:1 light petroleum/Et_2O) to afford pure **5** (1.25 g, 92%) as a colourless solid. Crystallization from light petroleum/Et_2O gave colourless crystals, mp 64 °C, $[\alpha]_D^{23} = -194.6$ (c 0.5, $CHCl_3$), $R_f = 0.30$ (1:1 light petroleum/Et_2O). IR, NMR (^1H and ^{13}C) and HRMS data were in full agreement with previously reported values [4].

Procedure B. Compound **16a** was converted to lactone **5** (in a yield of 94%) according to the reported procedure [4].

*4.1.6. 2,3-Dideoxy-D-arabino-hept-2-eno-1,5-lactone (**9**)*

A solution of **5** (0.08 g, 0.18 mmol) and $FeCl_3$ (0.058 g, 0.36 mmol) in anhydrous CH_2Cl_2 (8 mL) was stirred at room temperature for 0.5 h. An additional amount of $FeCl_3$ (0.015 g, 0.09 mmol) was added, the mixture was stirred at room temperature for 20 h and then evaporated. The residue was digested with dry CH_3CN (2 × 4 mL), organic phase was filtered and evaporated. The residue was purified on two columns of flash silica (9:1 EtOAc/MeOH) to afford pure **9** (0.029 g, 92%) as a colourless glassy solid. Recrystallization from 2-propanol gave a white powder, mp 104–106 °C, $[\alpha]_D^{23} = -162.6$ (*c* 0.5, MeOH), $R_f = 0.33$ (9:1 EtOAc/MeOH). IR (KBr): ν_{max} 3419, 3336, 1719, 1627. ^{1}H NMR (400 MHz, D_2O): δ 3.81 (dd, 1 H, $J_{7a,7b} = 12.3$, $J_{6,7a} = 2.7$ Hz, H-7a), 3.92 (dd, 1 H, $J_{6,7b} = 5.2$, $J_{7a,7b} = 12.3$ Hz, H-7b), 4.08 (ddd, 1 H, $J_{6,7a}$=2.7, $J_{6,7b} = 5.2$, $J_{5,6} = 8.1$ Hz, H-6), 6.21 (d, 1 H, $J_{2,3} = 9.7$ Hz, H-2), 7.25 (dd, 1 H, $J_{2,3} = 9.7$, $J_{3,4} = 6.1$ Hz, H-3). ^{13}C NMR (100 MHz, D_2O): δ 61.5 (C-4), 62.8 (C-7), 71.2 (C-6), 81.9 (C-5), 124.5 (C-2), 148.8 (C-3), 169.2 (C-1). HRMS (ESI): *m/z* 213.0152 (M^++K), calcd for $C_7H_{10}KO_5$: 213.0160.

*4.1.7. 5-O-Acetyl-6-O-benzoyl-3-O-benzyl-1,2-O-isopropylidene-α-D-glucofuranose (**20**)*

A solution of **17** (2.9 g, 8.28 mmol) in 60% aq AcOH (60 mL) was vigorously stirred at room temperature for 24 h. The mixture was evaporated by co-distillation with toluene to remove traces of acid and water, and the resulting residue was dried overnight under high vacuum. To a stirred solution of crude **18** and BzCl (0.96 mL, 8.28 mmol) in anhydrous CH_2Cl_2 (75 mL) was added DMAP (3.033 g, 24.83 mmol) and the mixture was stirred at room temperature for 1 h. Acetyl chloride (0.88 mL, 12.42 mmol) and DMAP (2.6 g, 21.28 mmol) were finally added to the mixture and the stirring at room temperature was continued for the next 1 h. Reaction mixture was poured in 10% aq NaCl (300 mL) and extracted with CH_2Cl_2 (3 × 40 mL). Combined organic phases were washed with 10% aq NaCl (200 mL), dried and evaporated and the resulting residue was purified on two columns of flash silica (3:7 Et_2O/light petroleum). Pure product **20** (2.6 g, 69%) was isolated as a colourless glassy solid, which crystallizes from Et_2O/hexane as colourless needles, mp 96–97 °C, lit. [12] mp 95–96 °C, $[\alpha]_D = -64.2$ ($CHCl_3$, *c* 0.5), lit. [12] $[\alpha]_D = -67.1$ ($CHCl_3$, *c* 1.22), $R_f = 0.25$ (3:1 light petroleum/Et_2O). IR (film): ν_{max} 1748, 1724, 1602, 1585, 1165. ^{1}H NMR (250 MHz, $CDCl_3$): δ 1.35 and 1.51 (2 × s, 3 H each, Me_2C), 1.91 (s, 3 H, *Me*CO), 3.99 (d, 1 H, $J_{3,4} = 3.1$ Hz, H-3), 4.34–4.52 (m, 3 H, CH_2Ph, H-4, H-6a), 4.66 (d, 1 H, $J_{1,2} = 3.7$ Hz, H-2), 4.66 (d, 1 H, J_{gem} = 11.7 Hz, CH_2Ph), 4.92 (dd, 1 H, $J_{5,6b} = 2.1$, $J_{6a,6b} = 12.3$ Hz, H-6b), 5.50 (ddd, 1 H, $J_{5,6b} = 2.1$, J = 5.0, J = 8.5 Hz, H-5), 5.96 (d, 1 H, $J_{1,2} = 3.7$ Hz, H-1), 7.28–8.18 (m, 10 H, Ph). ^{13}C NMR (62.5 MHz, $CDCl_3$): δ 20.8 (*Me*CO), 26.2 and 26.8 (Me_2C), 63.7 (C-6), 68.5 (C-5), 71.9 (CH_2Ph), 77.8

(C-4), 80.4 (C-3), 81.6 (C-2), 105.2 (C-1), 111.9 (Me_2C), 128.0, 128.2, 128.3, 128.6, 129.6, 130.0, 132.9, 136.8 (Ph), 166.1 (*C*OPh), 169.5 (Me*C*O). HRMS (ESI): *m/z* 495.1410 (M^++K), calcd for $C_{25}H_{28}KO_8$: 495.1416.

*4.1.8. 6-O-Acetyl-7-O-benzoyl-4-O-benzyl-2,3-dideoxy-D-arabino-hept-2-eno-1,5-lactone (**6**)*

To a solution of **20** (1.25 g, 2.74 mmol) in CH_2Cl_2 (12.5 mL) which was vigorously stirred at 0 °C was added 90% aq TFA (25 mL). After 0.5 h the cooling was stopped and the reaction continued at room temperature for additional 2 h. The solution was evaporated by co-distillation with toluene to remove the traces of acid and water. A small amount of the residue was purified (for characterization purposes only) by flash column chromatography (7:3 Et_2O/light petroleum). Colourless solid, mp 130–132 °C (from CH_2Cl_2/hexane), $[\alpha]_D$ = +62.4 ($CHCl_3$, *c* 0.5), R_f = 0.31 (7:3 Et_2O/light petroleum). Anomeric ratio: β/α = 2:1 (from ^{1}H NMR). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.95 (s, 3 H, *Me*CO), 3.50 (bs, 2 H, 2 × OH), 3.96–5.52 (overlapped H-2, H-3, H-4, H-5, 2 × H-6 and CH_2Ph), 5.23 (s, 1 H, H-1β), 5.54 (d, 1 H, $J_{1,2}$=4.0 Hz, H-1α), 7.29−8.07 (m, 10 H, Ph). ^{13}C NMR (100 MHz, $CDCl_3$): δ 21.23 (*Me*CO), 64.2 (C-6β), 64.3 (C-6α), 69.5, 69.8, 72.3, 73.0, 74.8, 77.0, 77.3, 79.2, 81.6, 82.8, 97.1 (C-1α), 104.0 (C-1β), 128.3, 128.4, 128.7, 128.72, 128.8, 129.0, 130.0, 130.1, 133.4, 136.6, 137.4 (Ph), 166.9 (*C*OPh), 170.3 (Me*C*O). HRMS (ESI): *m/z* 434.1802 (M^++NH_4), calcd for $C_{18}H_{19}Cl_3NO_8$: 434.1809. To a cooled (0 °C) solution of crude intermediate **21** in EtOAc (15 mL), were successively added H_2O (15 mL) and H_5IO_6 (1.25 g, 5.49 mmol). After 0.5 h the cooling bath was removed and the reaction continued at room temperature for additional 3.5 h. The mixture was poured to 10% aq NaCl (40 mL) and extracted with EtOAc (3 × 25 mL). The combined organic phases were washed with 10% aq NaCl (30 mL), then were dried and evaporated, and the resulting residue was dried under high vacuum for 2 h. To the solution of crude **22** in dry MeOH (100 mL) was added ECMP (1.15 g, 3.3 mmol) and the resulting mixture was stirred at room temperature for 20 h. The solution is evaporated and the resulting residue was purified by flash chromatography (3:2→1:1 light petroleum/Et_2O). An inseparable 1:1 mixture (^{1}H NMR) of ethyl and methyl esters (**23** and **24**) was obtained (0.6 g), R_f = 0.47 (1:1 light petroleum/Et_2O). The mixture was used in the next step without separation. A solution of purified mixture of **23** and **24** (0.315 g, 0.69 mmol) and TsOH·H_2O (0.006 g, 0.03 mmol) in dry CH_2Cl_2 (31 mL) was stirred at room temperature for 20 h. the reaction solution is evaporated, the resulting residue was purified by flash chromatography (2:3 light petroleum/Et_2O). Pure **6** (0.225 g, 79%, 38% from **20**) as a syrup that crystallized from a mixture of Et_2O/traces CH_2Cl_2/hexane as a colourless solid, mp 100 °C, $[\alpha]_D$ = −222.2 ($CHCl_3$, *c* 0.5), R_f = 0.30 (3:2 light petroleum/Et_2O). IR (KBr): ν_{max} 1741, 1728, 1602, 1585, 1177. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.08 (s, 3 H, *Me*CO), 4.05 (dd, 1 H, $J_{3,4}$ = 5.6, $J_{4,5}$ = 2.7

Hz, H-4), 4.51 and 4.61 (2 × d, 2 H, J_{gem} = 11.8 Hz, CH_2Ph), 4.54 (dd, 1 H, $J_{6,7a}$ = 4.2, $J_{7a,7b}$ = 12.6 Hz, H-7a), 4.66 (dd, 1 H, $J_{4,5}$ = 2.6, $J_{5,6}$ = 8.8 Hz, H-5), 5.03 (dd, 1 H, $J_{7a,7b}$ = 12.5, $J_{6,7a}$ = 2.3 Hz, H-7b), 5.62 (ddd, 1 H, $J_{6,7b}$ = 2.3, $J_{6,7a}$ = 4.2, $J_{5,6}$=8.8 Hz, H-6), 6.24 (d, 1 H, $J_{2,3}$ = 9.8 Hz, H-2), 7.03 (dd, 1 H, $J_{2,3}$ = 9.8, $J_{3,4}$=5.6 Hz, H-3), 7.28–8.07 (m, 10 H, Ph). ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.8 (*Me*CO), 61.8 (C-7), 64.0 (C-4), 68.5 (C-6), 71.2 (*C*H_2Ph), 76.9 (C-5), 124.4 (C-2), 128.2, 128.3, 128.34, 128.6, 129.5, 129.6, 133.1, 136.6 (Ph), 142.4 (C-3), 161.6 (C-1), 166.0 (*C*OPh), 169.3 (Me*C*O). HRMS (ESI): *m/z* 449.0990 (M^++K), calcd for $C_{23}H_{22}KO_7$: 449.0997.

*4.1.9. 6-O-Acetyl-7-O-benzoyl-2,3-dideoxy-D-arabino-hept-2-eno-1,5-lactone (**10**)*

To a stirred solution of **6** (0.32 g, 0.78 mmol) in CH_2Cl_2 (18 mL) was added 0.66% solution of $TiCl_4$ (2.61 mL, 1.56 mmol) in dry CH_2Cl_2. After 24 h, the reaction mixture was poured into 10% aq $NaHCO_3$ (60 mL) and the resulting suspension extracted with CH_2Cl_2 (4 × 20 mL). Organic phases were washed with 10% aq NaCl (50 mL), dried and evaporated, and the resulting residue was purified by flash chromatography (Et_2O). Pure **10** (0.176 g, 70%) was obtained, as a white solid, mp 118–120 °C. Recrystallization from CH_2Cl_2/hexane yielded a white powder, mp 124 °C, $[\alpha]_D^{23}$ = −32.0 (*c* 0.5, $CHCl_3$), lit. [6] $[\alpha]_D$ = −29.5 (*c* 0.45, $CHCl_3$), R_f = 0.30 (Et_2O). IR (KBr): ν_{max} 3420, 1743. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.12, (s, 3 H, *Me*CO), 3.69 (br s, 1 H, OH), 4.25 (t, 1 H, *J* = 5.0 Hz, H-4), 4.52–4.63 (m, 2 H, H-5 and H-7a), 4.95 (dd, 1 H, $J_{7a,7b}$ = 12.5, $J_{6,7b}$=2.0 Hz, H-7b), 5.52 (ddd, 1 H, $J_{6,7b}$ = 2.2, $J_{6,7a}$ = 4.9, $J_{5,6}$ = 9.5 Hz, H-6), 6.15 (d, 1 H, $J_{2,3}$ = 9.7 Hz, H-2), 7.04 (dd, 1 H, $J_{2,3}$ = 9.7, $J_{3,4}$=3.6 Hz, H-3), 7.34–8.08 (m, 5 H, Ph). ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.9 (*Me*CO), 59.3 (C-4), 62.5 (C-7), 69.1 (C-6), 77.6 (C-5), 123.2 (C-2), 128.5, 129.6, 129.7, 133.3 (Ph), 143.7 (C-3), 162.4 (C-1), 166.3 (*C*OPh), 170.8 (Me*C*O). HRMS (ESI): *m/z* 343.0787 (M^++Na), calcd for $C_{16}H_{16}NaO_7$: 343.0788.

*4.1.10. (−)-Cleistenolide (**1**)*

Procedure A. To a stirred suspension of **10** (0.176 g, 0.55 mmol) in Ac_2O (5 mL, 10.6 mmol) was added $TsOH·H_2O$ (0.005 g, 0.026 mmol). The mixture was stirred at room temperature for 4 h and then evaporated by co-distillation with toluene to remove the traces of Ac_2O. The residue was purified by flash column chromatography on silica gel (1:2 light petroleum/Et_2O) to yield pure **1** (0.19 g, 95%) in the form of a white solid, mp 135 °C, lit. [8] mp 133–134 °C, $[\alpha]_D$ = −212.2 (*c* 0.5, $CHCl_3$), or $[\alpha]_D$ = −212.4 (*c* 0.5, CH_2Cl_2), lit. [8] $[\alpha]_D$ = −164.6 (*c* 0.48, CH_2Cl_2). Recrystallization from Et_2O/hexane gave white needles, mp 138 °C, R_f = 0.36 (3:7 light petroleum/Et_2O).

Procedure B. To a stirred solution of **5** (0.05 g, 0.11 mmol) and BzBr (0.017 mL, 0.14 mmol) in dry CH_2Cl_2 (5 mL) was added $FeCl_3$ (0.005 g, 0.03 mmol) and the mixture was stirred at room temperature for 2 h. After that, to the mixture was added AcBr (0.16 mL, 2.2 mmol) and the reaction was continued at room temperature for additional 20 h. Reaction mixture was diluted with CH_2Cl_2 (10 mL), poured in 10% aq $NaHCO_3$ (60 mL) and extracted with CH_2Cl_2 (2 × 15 mL). The organic phases were combined, washed with 10% aq NaCl (30 mL), dried and evaporated, and the resulting residue was purified by flash column chromatography (2:1 light petroleum/Et_2O) to afford pure **1** (0.030 g, 74%) as a colourless solid, mp 135 °C, lit. [8] mp 133–134 °C. Recrystallization from Et_2O/hexane gave colourless needles, mp 138 °C, $[\alpha]_D = -212.2$ (*c* 0.5, $CHCl_3$), lit. [8] $[\alpha]_D = -164.6$ (*c* 0.5, CH_2Cl_2), $R_f = 0.36$ (7:3 Et_2O/light petroleum). IR (KBr): ν_{max} 1752, 1732, 1601, 1183. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.06 and 2.11 (2 × s, 3 H each, 2 × *Me*CO), 4.54 (dd, 1 H, $J_{6,7a} = 4.5$, $J_{7a,7b} = 12.5$ Hz, H-7a), 4.82 (dd, 1 H, $J_{5,6} = 9.6$, $J_{4,5} = 2.7$ Hz, H-5), 4.94 (dd, 1 H, $J_{6,7b} = 2.4$, $J_{7a,7b} = 12.5$ Hz, H-7b), 5.44 (dd, 1 H, $J_{3,4} = 6.1$, $J_{4,5} = 2.7$ Hz, H-4), 5.53 (ddd, 1 H, $J_{5,6} = 9.6$, $J_{6,7a} = 4.5$, $J_{6,7b} = 2.4$ Hz, H-6), 6.30 (d, 1 H, $J_{2,3} = 9.7$ Hz, H-2), 7.01 (dd, 1 H, $J_{2,3} = 9.7$, $J_{3,4} = 6.1$ Hz, H-3), 7.42–8.08 (m, 5 H, Ph). ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.5 and 20.7 (2 × *Me*CO), 59.8 (C-4), 62.0 (C-7), 67.7 (C-6), 75.5 (C-5), 125.4 (C-2), 128.5, 129.6, 129.7, 133.3 (Ph), 139.7 (C-3), 161.1 (C-1), 166.0 (*C*OPh), 169.5 and 169.9 (2 × Me*C*O). HRMS (ESI): *m/z* 385.0897 (M^++Na), calcd for $C_{18}H_{18}NaO_8$: 385.0894.

*4.1.11. 4,6-Di-O-acetyl-7-O-(3-methoxybenzoyl)-2,3-dideoxy-D-arabino-hept-2-eno-1,5-lactone (**2**)*

To a stirred solution of **9** (0.1 g, 0.57 mmol) in a mixture of anhydrous Py (2 mL) and CH_2Cl_2 (2 mL) was first added 3-methoxybenzoyl chloride (0.08 mL, 0.58 mmol) and the mixture was stirred at room temperature for 4 h. Acetic anhydride (4 mL) was added to the solution and the stirring at room temperature was continued for the next 20 h. The mixture was evaporated by co-distillation with toluene to remove traces of Py and Ac_2O. The residue was suspended in water (70 mL) and extracted with CH_2Cl_2 (3 × 20 mL). The combined organic solutions were dried and evaporated, and the remaining crude mixture was purified by flash column chromatography (9:1 toluene/Me_2CO) to give pure **2** (0.152 g, 67%) as a colourless glassy solid. Recrystallization from 2-propanol gave a white powder, mp 85 °C, $[\alpha]_D^{23} = -187.8$ (*c* 0.5, $CHCl_3$), $R_f = 0.30$ (9:1 toluene/Me_2CO). IR (KBr): ν_{max} 1750, 1734, 1636, 1604, 1588, 1182, 1153. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.05 and 2.10 (2 × s, 3 H each, 2 × *Me*CO), 3.86 (s, 3 H, CH_3O), 4.52 (dd, 1 H, $J_{7a,7b} = 12.5$, $J_{6,7a} = 4.6$ Hz, H-7a), 4.80 (dd, 1 H, $J_{4,5} = 2.5$, $J_{5,6} = 9.6$ Hz, H-5), 4.92 (dd, 1 H, $J_{7a,7b} = 12.5$, $J_{6,7b} = 2.1$ Hz, H-7b), 5.43 (dd, 1 H, $J_{4,5} = 2.6$, $J_{3,4} = 6.0$ Hz, H-4), 5.52 (ddd, 1 H, $J_{6,7b} = 2.1$, $J_{6,7a} = 4.6$, $J_{5,6} = 9.6$ Hz, H-6), 6.29 (d, 1 H, $J_{2,3} = 9.7$ Hz, H-2), 7.00 (dd, 1 H, $J_{2,3} = 9.7$, $J_{3,4} = 6.1$ Hz, H-

3), 7.09–7.64 (m, 4 H, MeO*Ph*). ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.5 and 20.7 (2 × *Me*CO), 55.5 (OMe), 59.7 (C-4), 62.2 (C-7), 67.6 (C-6), 75.5 (C-5), 125.3 (C-2), 114.4, 119.6, 122.0, 129.5, 130.9, 159.6 (MeO*Ph*), 139.7 (C-3), 161.1 (C-1), 165.9 (Ar*C*O), 169.5, 169.9 (2 × Me*C*O). HRMS (ESI): *m/z* 431.0732 (M^++K), calcd for $C_{19}H_{20}KO_9$: 431.0739.

4.1.12. 4,6-Di-O-acetyl-7-O-(2,4,6-trichlorobenzoyl)-2,3-dideoxy-D-arabino-hept-2-eno-1,5-lactone ***(3)***

To a stirred solution of **9** (0.05 g, 0.29 mmol) in a mixture of anhydrous Py (1 mL) and CH_2Cl_2 (1 mL) was first added 2,4,6-trichlorobenzoyl chloride (0.04 mL, 0.26 mmol) and the mixture was stirred at room temperature for 4 h. Acetic anhydride (2 mL) was added to the solution and the stirring at room temperature was continued for the next 20 h. The mixture was evaporated by co-distillation with toluene and the residue was purified on a column of flash silica (1:1 Et_2O/light petroleum) to give pure **3** (0.114 g, 85%) as a colourless solid. Recrystallization from CH_2Cl_2/hexane gave a white powder, mp 134 °C, $[\alpha]_D^{23} = -193.6$ ($CHCl_3$, *c* 0.5), $R_f = 0.30$ (1:1 Et_2O/light petroleum). IR (KBr): ν_{max} 1747, 1631, 1580, 1548, 1193, 1161. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.07 and 2.09 (2 × s, 3 H each, 2 × *Me*CO), 4.61 (dd, 1 H, $J_{7a,7b} = 12.5$, $J_{6,7a} = 2.5$ Hz, H-7a), 4.80 (dd, 1 H, $J_{4,5} = 1.7$, $J_{5,6} = 9.5$ Hz, H-5), 5.00 (bd, 1 H, $J_{7a,7b} = 12.5$ Hz, H-7b), 5.39 (dd, 1 H, $J_{4,5} = 1.8$, $J_{3,4} = 6.0$ Hz, H-4), 5.44 (bd, 1 H, $J_{5,6} = 9.5$ Hz, H-6), 6.27 (d, 1 H, $J_{2,3} = 9.7$ Hz, H-2), 6.99 (dd, 1 H, $J_{2,3} = 9.6$, $J_{3,4} = 6.1$ Hz, H-3), 8.36 (s, 2 H, 2,4,6-trichlorophenyl). ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.4 and 20.7 (2 × *Me*CO), 59.7 (C-4), 62.8 (C-7), 67.4 (C-6), 74.9 (C-5), 125.3 (C-2), 128.1, 131.6, 132.6, 136.5 (2,4,6-trichlorophenyl), 139.7 (C-3), 161.0 (C-1), 163.5 (Ar*C*O), 169.4 and 169.9 (2 × Me*C*O). HRMS (ESI): *m/z* 482.0169 (M^++NH_4), calcd for $C_{18}H_{19}Cl_3NO_8$: 482.0171.

*4.1.13. 4,6-Di-O-acetyl-7-O-cinnamoyl-2,3-dideoxy-D-arabino-hept-2-eno-1,5-lactone (**4**)*

To a stirred solution of **9** (0.08 g, 0.46 mmol) in a mixture of anhydrous Py (2.5 mL) and CH_2Cl_2 (2.5 mL) was first added *trans*-cinnamoyl chloride (0.095 g, 0.57 mmol) and the mixture was stirred at room temperature for 2 h. Acetic anhydride (5 mL) was added to the mixture and the stirring at room temperature was continued for the next 18 h. The solution was evaporated by co-distillation with toluene. The residue was suspended in H_2O (80 mL) and extracted with CH_2Cl_2 (3 × 20 mL). The combined organic phases were washed with 10% aq NaCl (50 mL) dried and evaporated. Oily residue was purified by flash column chromatography (3:2 Et_2O/light petroleum) to afford pure **4** (0.084 g, 47%) as a colourless solid. Recrystallization from Et_2O/light petroleum

gave a white powder, mp 110 °C, $[\alpha]_D^{23} = -212.8$ ($CHCl_3$, *c* 0.5), $R_f = 0.34$ (3:2 Et_2O/light petroleum). IR (KBr): ν_{max} 1750, 1736, 1642, 1578, 1180. ^1H NMR (400 MHz, $CDCl_3$): δ 2.08 and 2.10 (2 × s, 3 H each, 2 × *Me*CO), 4.44 (dd, 1 H, $J_{7a,7b} = 12.6$, $J_{6,7a} = 3.9$ Hz, H-7a), 4.78–4.87 (m, 2 H, H-5 and H-7b), 5.39–5.49 (m, 2 H, H-4 and H-6), 6.30 (d, 1 H, $J_{2,3} = 9.7$ Hz, H-2), 6.46 (d, 1 H, $J_{2',3'} = 16.0$ Hz, H-2′), 7.02 (dd, 1 H, $J_{2,3} = 9.6$, $J_{3,4} = 6.1$ Hz, H-3), 7.35–7.61 (m, 5 H, Ph), 7.73 (d, 1 H, $J_{2',3'} = 16.0$ Hz, H-3′). ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.5 and 20.7 (2 × *Me*CO), 59.8 (C-4), 61.5 (C-7), 67.7 (C-6), 75.3 (C-5), 117.2 (C-2′), 125.4 (C-2), 128.2, 128.9, 130.6, 134.2 (Ph), 139.7 (C-3), 145.8 (C-3′), 161.2 (C-1), 166.1 (C-1′), 169.5 and 169.9 (2 × Me*C*O). HRMS (ESI): *m/z* 411.1045 (M^++Na), calcd for $C_{20}H_{20}NaO_8$: 411.1050.

4.1.14. 4,6,7-Tri-O-benzoyl-2,3-dideoxy-D-arabino-hept-2-eno-1,5-lactone (7)

To a stirred solution of **9** (0.04 g, 0.23 mmol) in a mixture of anhydrous Py (1.5 mL) and CH_2Cl_2 (1.5 mL) was added slowly benzoyl chloride (0.16 mL, 1.38 mmol) in portions. The mixture was stirred at room temperature for 3.5 h, and the mixture was evaporated by co-distillation with toluene to remove traces of Py. The residue was dissolved in CH_2Cl_2, absorbed on flash silica and purified by flash column chromatography (11:9 Et_2O/light petroleum). Pure product **7** (0.096 g, 86%) was obtained as a turbid oil, which crystallizes from CH_2Cl_2/hexane as a white powder, mp 120 °C, $[\alpha]_D^{23} = -276.8$ ($CHCl_3$, *c* 1), $R_f = 0.35$ (3:7 EtOAc/light petroleum), or $R_f = 0.39$ (3:2 Et_2O/light petroleum). IR (film): ν_{max} 1726, 1601, 1585, 1177. ^1H NMR (400 MHz, $CDCl_3$): δ 4.78 (dd, 1 H, $J_{6,7a} = 4.6$, $J_{7a,7b} = 12.5$ Hz, H-7a), 5.08 (dd, 1 H, $J_{6,7b} = 2.2$, $J_{7a,7b} = 12.6$ Hz, H-7b), 5.12 (dd, $J_{4,5} = 2.4$, $J_{5,6} = 9.2$ Hz, H-5), 5.66 (dd, 1 H, $J_{3,4} = 6.0$, $J_{4,5} = 2.5$ Hz, H-4), 5.93 (m, 1 H, H-6), 6.35 (d, 1 H, $J_{2,3} = 9.7$ Hz, H-2), 7.23 (dd, 1 H, $J_{2,3} = 9.7$, $J_{3,4} = 6.0$ Hz, H-3), 7.35–8.04 (m, 15 H, Ph). ^{13}C NMR (100 MHz, $CDCl_3$): 61.0 (C-4), 62.2 (C-7), 68.5 (C-6), 75.9 (C-5), 125.3 (C-2), 128.36, 128.4, 128.5, 128.8, 129.5, 129.6, 129.8, 133.2, 133.5, 133.8 (Ph), 140.2 (C-3), 161.2 (C-1), 164.8, 165.2, 165.9 (3 × Ph*C*=O). HRMS (ESI): *m/z* 525.0948 (M^++K), calcd for $C_{28}H_{22}KO_8$: 525.0946.

*4.1.15. 4,6,7-Tri-O-(3-methoxybenzoyl)-2,3-dideoxy-D-arabino-hept-2-eno-1,5-lactone (**8**)*

To a stirred solution of **9** (0.04 g, 0.23 mmol) in a mixture of anhydrous Py (1.5 mL) and CH_2Cl_2 (1.5 mL) was slowly added 3-methoxybenzoyl chloride (0.2 mL, 1.38 mmol). The mixture was stirred at room temperature for 3.5 h, and the mixture was evaporated by co-distillation with toluene to remove traces of Py. The residue was purified on two columns of flash silica (2:1 Et_2O/light petroleum). Pure product **8** (0.117 g, 88%) was obtained in the form of turbid syrup,

$[\alpha]_D^{23}$ = −242.4 ($CHCl_3$, *c* 1), R_f = 0.33 (17:3 toluene/EtOAc) or R_f = 0.31 (7:3 Et_2O/light petroleum). IR (film): ν_{max} 1731, 1601, 1489, 1182. ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.77 (dd, 1 H, $J_{6,7a}$ = 4.5, $J_{7a,7b}$ = 12.7 Hz, H-7a), 5.03 (dd, 1 H, $J_{6,7b}$ = 1.7, $J_{7a,7b}$ = 12.7 Hz, H-7b), 3.78, 3.81 and 3.86 (3 × s, 9 H, 3 × OMe), 5.14 (dd, $J_{4,5}$ = 2.4, $J_{5,6}$ = 9.1 Hz, H-5), 5.63 (dd, 1 H, $J_{3,4}$ = 6.0, $J_{4,5}$ = 2.2 Hz, H-4), 5.91 (m, 1 H, H-6), 6.35 (d, 1 H, $J_{2,3}$ = 9.5 Hz, H-2), 7.04–8.04 (m, 13 H, H-3 and Ph). ^{13}C NMR (100 MHz, $CDCl_3$): 55.38, 55.4 and 55.5 (3 × OMe), 61.2 (C-4), 62.4 (C-7), 68.6 (C-6), 75.9 (C-5), 125.3 (C-2), 114.2, 114.2 and 114.25 (3 × C-2′), 119.7, 120.0, 120.4 (3 × C-4′), 122.0, 122.3 (C-6′), 129.5, 129.6 (C-5′), 140.3 (C-3), 159.5, 159.57, 159.6 (C-3′) 161.2 (C-1), 165.8, 165.2, 164.7 (3 × C=O). HRMS (ESI): *m/z* 615.1268 (M^++K), calcd for $C_{31}H_{28}KO_{11}$: 615.1263.

4.2. Antimicrobial activity

4.2.1. Test microorganisms

The compounds were tested against the panel of microbial strains belonging to the American Type Culture Collection reference strains, including the following: Gram-positive bacteria (*Staphylococcus aureus* ATCC 6538, *Staphylococcus epidermidis* ATCC 12228, *Bacillus cereus* ATCC 11778, and *Kocuria rhizophila* ATCC 9431), Gram-negative bacteria (*Pseudomonas aeruginosa* ATCC 9027, *Escherichia coli* ATCC 8739, *Salmonella enteritidis* ATCC 13076, and *Acinetobacter baumannii* ATCC 19606) and yeast *Candida albicans* (ATCC 10231). Bacterial strains were maintained on Nutrient Agar (NA) at 37 °C, while the yeast strain was grown on Sabouraud Dextrose Agar (SDA) at 30 °C at the Microbiology Laboratory (Department of Biology, Faculty of Sciences and Mathematics, University of Niš).

4.2.2. Screening of antimicrobial activity (microdilution method)

Antimicrobial activity was evaluated using a broth microdilution method in microtiter plates as described earlier [23]. Briefly, cell suspensions standardized to 0.5 McFarland (DEN-1, Biosan) turbidity were made using an overnight culture (18 h) of the test microorganisms. Stock solutions of the test compounds were made in pure DMSO and diluted with appropriate sterile broth (Sabouraud Dextrose or Mueller Hinton broth). The highest final concentration (10%, *v*/*v*) of DMSO needed to achieve complete dissolution of the compounds was confirmed not to affect either bacterial or fungal growth. After making doubling dilutions of the test substances in the concentration range 0.002–6.00 μmol/ml, the inoculum was added to all wells and the plates were incubated at 37 °C during 24 h in the case of bacteria or at 30 °C during 48 h for the yeast. Chloramphenicol and

nystatin served as positive controls. The bacterial growth was determined by adding 20 μl of 0.5% (*w*/*w*) triphenyltetrazolium chloride (TTC) aqueous solution. MIC was defined as the lowest concentration of the test compound that inhibited visible growth (red-coloured pellet on the bottom of the wells after the addition of TTC). All experiments were done in triplicate and repeated two times.

4.3. Statistical treatment

Hierarchical clustering and heatmap preparation were performed using the freeware Morpheus [24]. The proximity between two objects in the dendrograms was measured by the Euclidean distance. The average-linkage method was applied as the aggregation criterion. The number of object classes (groups of observations) was chosen based on the increase of within-group and between-group dissimilarities. The method was applied utilizing original variables (MIC values).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in online version.

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TITLE: Synthesis and antimicrobial activity of (−)-cleistenolide and analogues

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Highlights

- New synthesis of (−)-cleistenolide was achieved using the "chiral pool" approach
- The same approach was used to prepare several novel (−)-cleistenolide analogues
- Most analogues showed antimicrobial activity against some bacterial strains