Structure Activity Relationship Study of the Cleistriosides and Cleistetrosides for Antibacterial/Anticancer Activity

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Abstract

Two known cleistriosides and six known cleistetrosides were synthesized and evaluated for anticancer and antibacterial activity. This study, for the first time, reports anticancer activity and comprehensively the antibacterial activity for these oligosaccharide natural products. In addition, two new unnatural cleistetroside analogues were synthesized and tested. Biological activities for the ten oligosaccharides against *B. subtilis* were found to range between 4 and >64 μM, and for NCI-H460 human lung cancer epithelial cells between 7.5 and 90.9 μM. Similar activities were found for seven of the oligosaccharides against the NCI panel of 60 cell lines. The degree of acylation and location of the specific acetate groups had significant effects on the anticancer and antibacterial activity of both the cleistriosides and cleistetrosides.

Keywords

Cleistetroside; Cleistrioside; antibacterial; anticancer; Palladium-catalyzed glycosylation

As part of a high-throughput based search for new natural products with unique structures and interesting biological activity, two partially acetylated trisaccharide (Cleistrioside-5/-6, **1** and **2**) and six partially acetylated tetrasaccharide (Cleistetroside-2/-3/-4/-5/-6/-7, **3–8**) natural products were discovered (Figure 1) from the leaves and bark of trees with a folk medicinal tradition.[1,2,3] These dodecanyl tri- and tetra-rhamnoside structures with various degree of acylation were isolated from *Cleistopholis patens* and *glauca*.[1,2] The structures of the cleistriosides and cleistetrosides were assigned by detailed NMR analysis and later confirmed by total synthesis. The absolute and relative stereochemistry for one member of the cleistetrosides (**3**) was confirmed by two total synthetic efforts by Zhang[4] and Chen[5] in 2007 via a traditional carbohydrate approach. In 2010, we reported a divergent *de novo* asymmetric synthesis of both cleistriosides (**1** and **2**) as well as the six cleistetrosides (**3–8**), thus confirming the structures of the remaining members.[6]

Because of the limited amounts of natural isolates (1–3 mg), only two (cleistrioside-5 (**1**) and cleistetroside-2 (**3**)) of these eight oligo-*rhamno*-natural products were extensively evaluated for antimicrobial activity.[3] These antibacterial activities are outlined in Table 1,[3] which show quite potent activity across a broad range of organisms. Of particular note, is that both cleistrioside-5 (**1**) and cleistetroside-2 (**3**) showed significant activity against several oxacillin-resistant organisms (*e.g.*, *Staphylococcus aureus* ATCC 33591, Table 1).[3] Because of the rhamnan like structural motif of the cleistriosides and cleistetrosides, their antibacterial activity is assumed to occur via inhibition of mycobacterial cell wall synthesis. Similarly, the fact that the oligo-rhamnose motif is not found in mammalian cells makes them even more interesting as a potential antibiotic. [7]

Over the years, we have been interested in the *de novo* synthesis and study of partially acylated rhamnoside natural products.[8,9] Accordingly, the structurally novel cleistrioside and cleistetroside antibiotics inspired us to develop a divergent *de novo* asymmeric approach, which resulted in a reasonably efficient approach to all the members of these two classes of natural products (Scheme 1). While our *de novo* route to any particular member was not notably more efficient than the two traditional carbohydrate approaches to cleistetroside-2 (**3**),[4,5] the approach allows for the divergent synthesis of all eight oligo-rhamnosides (**1–8**) with the minimal use of protecting groups. The overall efficiency of the route is best exemplified in that it affords sufficient quantities of material for detailed biological investigations. Herein we describe our successful use of this divergent *de novo* approach to the eight natural products (**1–8**) along with two unnatural cleistetroside analogues (**9** and **10**) as well as their evaluation in antibacterial and cytotoxicity assays.

Previously, we demonstrated that the eight oligosaccharides (**1–8**) could be efficiently prepared via a divergent *de novo* route that derives all the sugar carbons from the achiral acylfuran **11**.[6] Key to the approach was the efficient synthesis of a common trisaccharide intermediate **17** (Scheme 2). In a three-step asymmetric protocol acylfuran was converted into pyranone glycosyl donor **12**,[10, 11] which in turn is converted to pyranone **13** via a Pd-catalyzed glycosylation (1:4/Pd$_2$(dba)$_3$•CHCl$_3$ to PPh$_3$). [12, 13, 14] A three-step post-glycosylation protocol was used to stereoselectively install the desired *rhamno*-stereochemistry and acetonide protecting groups. A subsequent glycosylation, reduction, acylation and dihydroxylation sequence provided disaccharide **15** followed by a regioselective glycosylation and chloroacylation gives the orthoganally protected trisaccharide **17**. This 14-step synthesis was accomplished with only two traditional (acetonide and chloroacetate) and two non-traditional (ketone and alkene) alcohol protecting groups.[15]

The enone portion of trisaccharide **17** was ready for further elaboration into the final *rhamno*-sugar required for **1** and **2**, in only 6 steps **17** was selectively converted into either trisaccharides **1** or **2**. By simply incorporating an additional glycosylation/post-glycosylation reaction sequence, along with traditional two deprotections, the final two *rhamno*-sugars of the cleistetrosides were installed onto **17**.[16] Thus in 9–11 steps, **17** was selectively converted into the six cleistetroside natural products **3** through **8**. In contrast to the isolation work, this synthetic approach provided significant enough pure material for detailed biological evaluation (*vide infra*), providing 5 mg of both cleistriosides and between 5 and 43 mg of the cleistetrosides (Scheme 3).

In addition to the six naturally occurring cleistetrosides, this route was amenable to the synthesis of two unnatural cleistetrosides analogues **9** and **10** (Scheme 4). The new route begins with the same trisaccharide intermediate **17**, which was reduced, chloroacylated and dihydroxylated to form diol **18**. A regioselective acylation, glycosylation, reduction, acylation and dihydroxylation converted **18** into tetrasaccharide **19** in 48% yield.[17,18]

Finally the two chloro-acetates ($(NH_2)_2CS/NaHCO_3/Bu_4NI$) and acetonide ($AcOH/H_2O$) were removed to give cleistetroside analogue **9** in 83% yield. Similarly, **18** can be converted into **20** via a regioselective acylation, glycosylation, reduction and dihydroxylation (58 % yield for 4 steps). Finally a per-acylation followed by a chloroacetate and acetonide deprotection gave cleistetroside analogue **10** in 93% yield.

With the ten target oligosaccharides in hand, we next set out to evaluate the structure-activity relationship (SAR) with respect to their activity against three different bacteria strains. We chose two *E. coli* strains (MG1655 and *imp*)[19] and one *B. subtilis* strain (JH642). Of the two *E. coli* strains the first (K-12 MG1655) is the wild-type Gram-(−) organism and the second *E. coli imp* (K-12 BAS901) is a Gram-(+)-like strain, which has been genetically modified (*imp*-4213) to increases the permeability of the outer membrane.[20] The *B. subtilis* strain (JH642) is a Gram-(+) organism. Consistent with the findings of Hu et al.,[3] we found both the cleistriosides and all the cleistetrosides were inactive against wild-type *E. coli*, which suggests that the oligosaccharides are not getting past the Gram-(−) organism cell wall. Further evidence along these lines came from the screening against the Gram-(+) like strain (*imp*), with three of the oligosaccharides showing improved activity. Improved MIC values were found for one of the natural trisaccharides (**2**, 32 μM) and one tetrasaccharide (**6**, 16 μM). Interestingly, of the two unnatural analogues (**9** and **10**), cleistetroside-new 1 (**9**, 16 μM) showed similar activity to **6**, which differs by only one acetate (R^4). As was suggested from the *E. coli* study, we found significantly greater activity against the *B. subtilis* strain, where the activities ranged from 4 to >64 μM.

Once again, cleistrioside-6 (**2**) was the more active of the two trisaccharide natural products, whereas significantly different trends were found for the tetrasaccharides **3–10**. Of the cleistetrosides, only two were inactive against *B. subtilis* (**8** and **10**, >64 μM), with the rest have MICs   8 μM. In particular, cleistetroside-2 and 6 (**3** and **7**) were found to be the most active (4 μM). As with the Gram-(+)-like *E. coli imp* the unnatural cleistetroside **9** was more active than **10** against *B. subtilis*.

Because the related acylated oligo-rhamnoside natural products were reported to possess anticancer activity,[21] we decided to test the set of ten oligosaccharides for their cytotoxicity against the human lung cancer cell line (NCI-H460). We found IC_{50}s for this class of natural products at similar concentrations (7.5–90.9 μM, Table 3) as the MIC for the Gram-(+) *B. subtilis* strain (4->64 μM, Table 2). Once again, of the two trisaccharides, cleistrioside-6 (**2**) was the most active. In contrast to the antibacterial activity, we found all the cleistetrosides to have significant cytotoxicity (< 20 μM). As we have found for anti-*B. subtilis* activity, we found cleistetrosides-6 (**7**) to be the most active (7.5 μM), with cleistetrosides-2 (**3**), showing similar activity (9.1 μM).

The fact that cleistetrosides-2 and -6 (**3** and **7**) are the most active and cleistetroside-7 (**8**) is the least active in both the anti-*B. subtilis* and anticancer screens, suggests the possibility that both the anti-*B. subtilis* and anticancer activities for this class of natural products result from the same molecular target or mode of action. To challenge this trend, we were inspired to prepare the two unnatural cleistetrosides (**9** and **10**), which had the C-4 acetate (R^1, Ac to H) in the third rhamnose sugar removed. The removal of the same acetate in the cleistriosides (R^4) was found to improve activity. Thus, cleistetroside-New 1 (**9**) is analogous to the quite potent cleistetroside-2 (**3**) and cleistetroside-New 2 (**10**) is analogous to least potent cleistetrosides 7 (**8**) with the C-4 acetates having been removed. The testing of compounds **9** and **10** clearly demonstrates the importance of the R^1 group to cleistetroside SAR. For instance, the removal of the acetate in cleistetroside-2 (**3**) had small but negative effect on its potency in both screens. In contrast, cleistetroside-New 2 (**10**) possessed dramatically different effects on its anti-*B. subtilis* activity (> 64 μM) as compared to

anticancer activity (<10 μM). Thus, with the synthesis and testing of only two new structures, we were able to find a compound that contradicted the correlation between anti-*B. subtilis* and anticancer activity. This, of course, could result from different modes of actions and/or different esterase activities in bacterial vs human cancer cells.

While there is not enough information to fully understand the effects of the acetate groups, clearly, the location of the individual acetate groups has a significant effect on both the anticancer and anti-*B. subtilis* activity. In contrast to the tetrasaccharides, this effect can easily be seen in cleistriosides-5 and 6 (**1** and **2**), where the simple migration of a C-4 acetate group on the terminal sugar in **1** to the C-3 position in **2** dramatically increases its cytotoxicity to both *B. subtilis* and cancer cells. Interestingly, the most active tetrasaccharides (**3**, **7** and **10**) have a C-4 acetate and are glycosylated at the C-3 position.

To gain a more complete understanding of the anticancer activities, the set of ten acylated oligo-rhamnosides (**1–10**) were screened against the NCI-panel of 60 cell lines. Of the ten compounds screened, three (**5**, **6** and **9**) did not pass the initial single dose assay. A representative subset (two from the nine tissue types) of these results is outlined in Table 4 (for the results of the full 60 cell lines see the Supporting Information). Since these experiments were run under different conditions (*e.g.*, time) and measure different parameters (*e.g.*, protein concentrations vs mitochondrial activity), it is difficult to directly compare the activities. However, some interesting trends can be seen as well as differences. For instance, the data from the NCI screen and our H460 MTT assay both show compounds **3**, **7** and **10** to have similar anticancer activity, with **8** being an outlier. Similarly, the unnatural tetrasaccharide **10** was significantly more active than **9**. In contrast to our data for NCI-H460, the NCI screen found tetrasaccharide **8** to be one of the most active against the full panel of cell lines, including the NCI-H460. This discrepancy presumably arises from the subtle differences in the two protocols.

In conclusion, we have demonstrated the utility of a *de novo* asymmetric synthesis for providing sufficient quantities of material for comprehensive SAR-type study. The route was amenable to the synthesis of unnatural oligosaccharide analogues for further SAR studies for both anticancer and antibacterial activities. It is worth noting that these efforts discover previously unreported anticancer activities for this class of acylated oligo-rhamnoside natural products. Further SAR studies along these lines are ongoing and will be reported in due course.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## Acknowledgments

ABBREVIATIONS

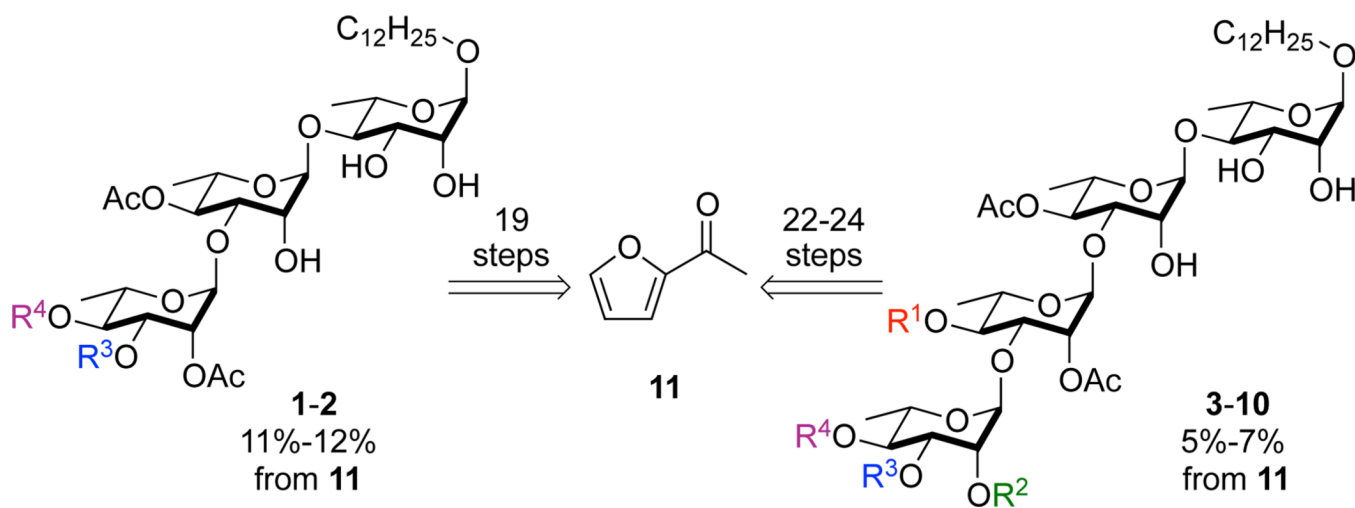|	
E. coli	Escherichia coli
B. subtilis	Bacillus subtilis

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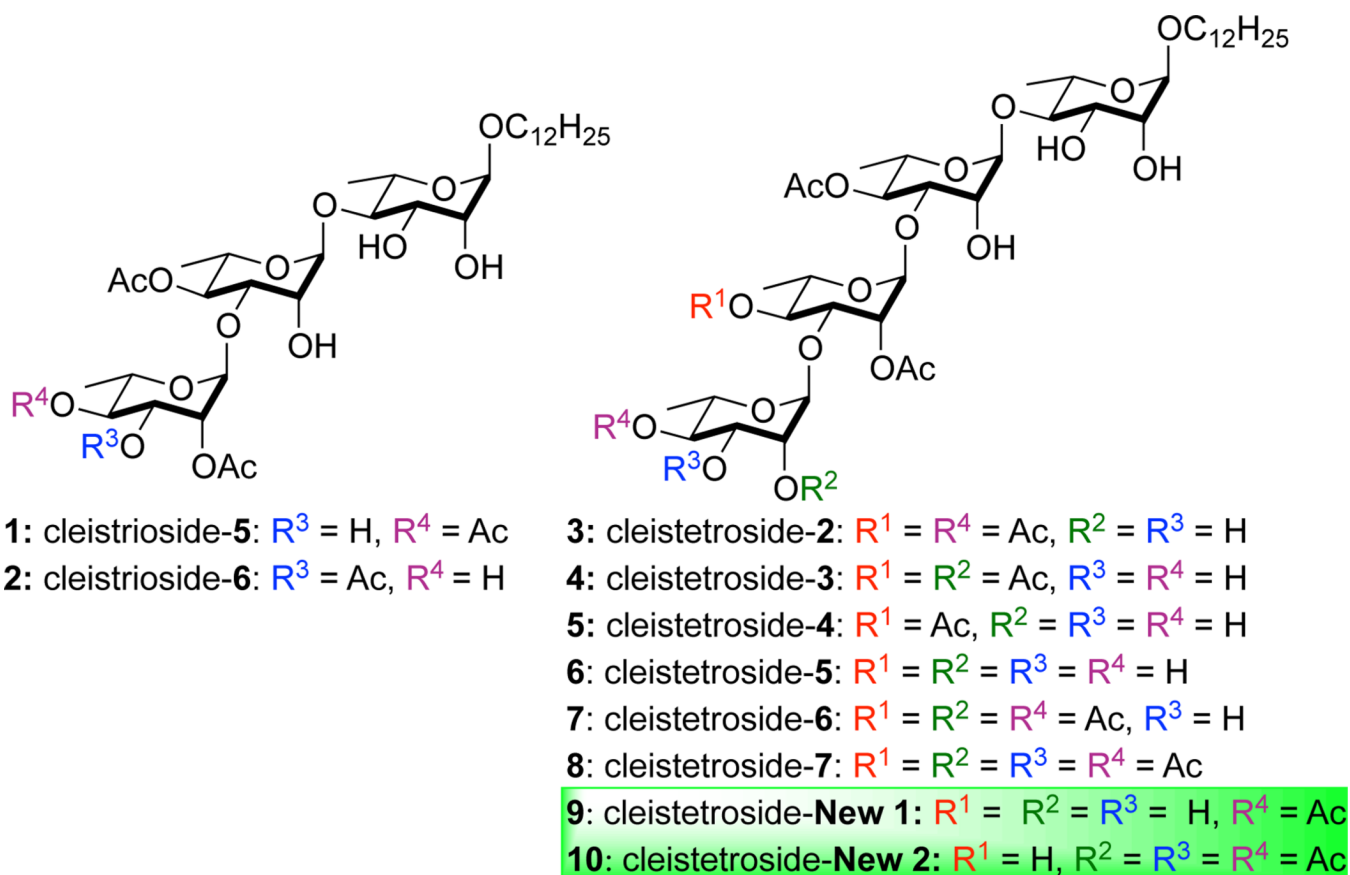
Figure 1.
Targeted cleistriosides and cleistetrosides

1: cleistrioside-**5**: R^3 = H, R^4 = Ac
2: cleistrioside-**6**: R^3 = Ac, R^4 = H

3: cleistetroside-**2**: R^1 = R^4 = Ac, R^2 = R^3 = H
4: cleistetroside-**3**: R^1 = R^2 = Ac, R^3 = R^4 = H
5: cleistetroside-**4**: R^1 = Ac, R^2 = R^3 = R^4 = H
6: cleistetroside-**5**: R^1 = R^2 = R^3 = R^4 = H
7: cleistetroside-**6**: R^1 = R^2 = R^4 = Ac, R^3 = H
8: cleistetroside-**7**: R^1 = R^2 = R^3 = R^4 = Ac
9: cleistetroside-**New 1**: R^1 = R^2 = R^3 = H, R^4 = Ac
10: cleistetroside-**New 2**: R^1 = H, R^2 = R^3 = R^4 = Ac

Scheme 1.
De Novo retrosynthetic approach to the cleistriosides and cleistetrosides
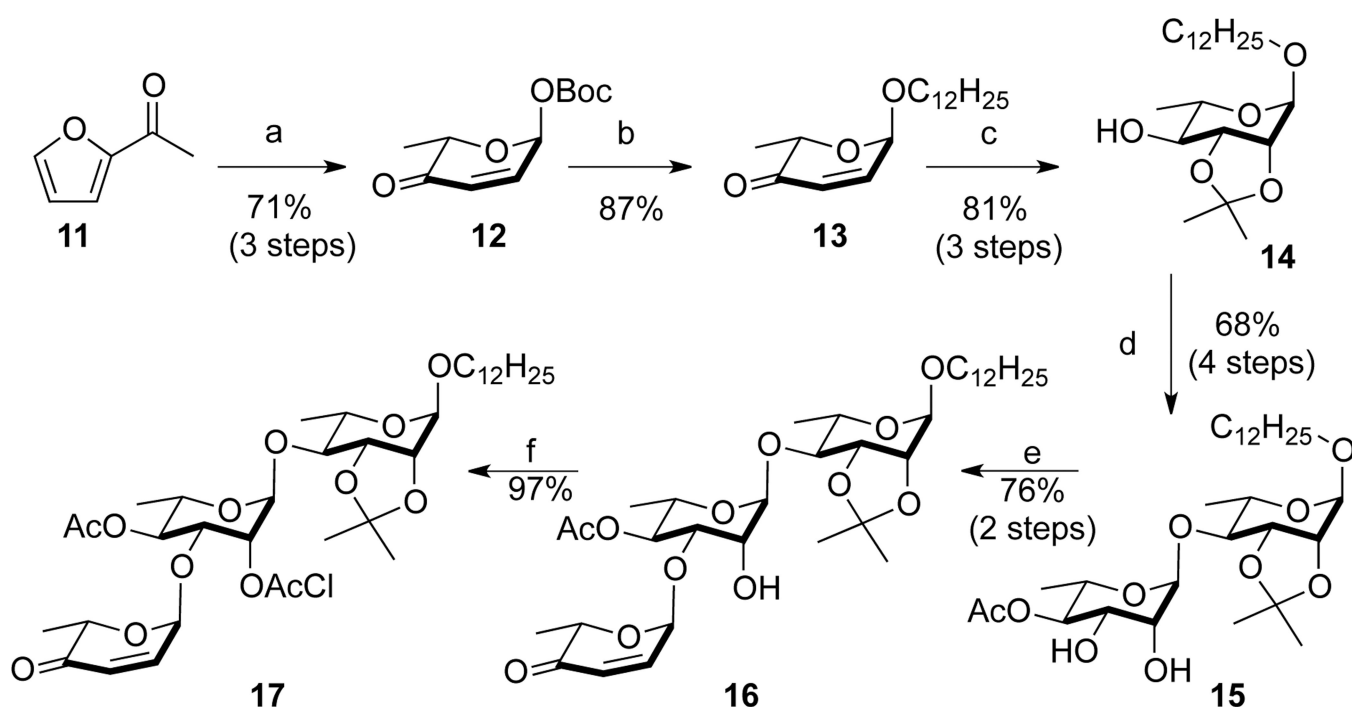
Reagents and conditions: (a) (i) Noyori (*S,S*), HCO₂Na (aq), 10 mol% CTAB, rt, 24 h; (ii) NBS, THF/H₂O (3:1), NaHCO₃, NaOAc•3H₂O, 0 °C, 1 h; (iii) Boc₂O, DMAP, CH₂Cl₂, −78 °C, 12 h; (b) HOCH₂(CH₂)₁₀CH₃, 5 mol % **Pd(PPh₃)₂,** CH₂Cl₂, 0 °C; (c) (i) NaBH₄, CeCl₃, CH₂Cl₂/MeOH, −78 °C, 2 h; (ii) OsO₄, NMO, *t*-BuOH/acetone, rt, 12 h; (iii) TsOH, 2,2-DMP, acetone, 0 °C, 4 h; (d) (i) **12**, 5 mol% **Pd(PPh₃)₂,** CH₂Cl₂, 0 °C, 2 h; (ii) NaBH₄, CH₂Cl₂/MeOH, −78 °C, 2 h; (iii) Ac₂O, Py, rt, 3 h; (iv) OsO₄, NMO, *t*-BuOH /acetone, rt, 12 h; (e) (i) *n*-Bu₂SnO, MeOH, reflux, 1h; (ii) **12**, 5 mol% **Pd(PPh₃)₂,** CH₂Cl₂, 0 °C, 2 h; (f) (ClAc)₂O, Py, 3 h. **Pd(PPh₃)₂** = Pd₂(dba)₃•CHCl₃/4PPh₃

Scheme 2.
Approach to the intermediate 17
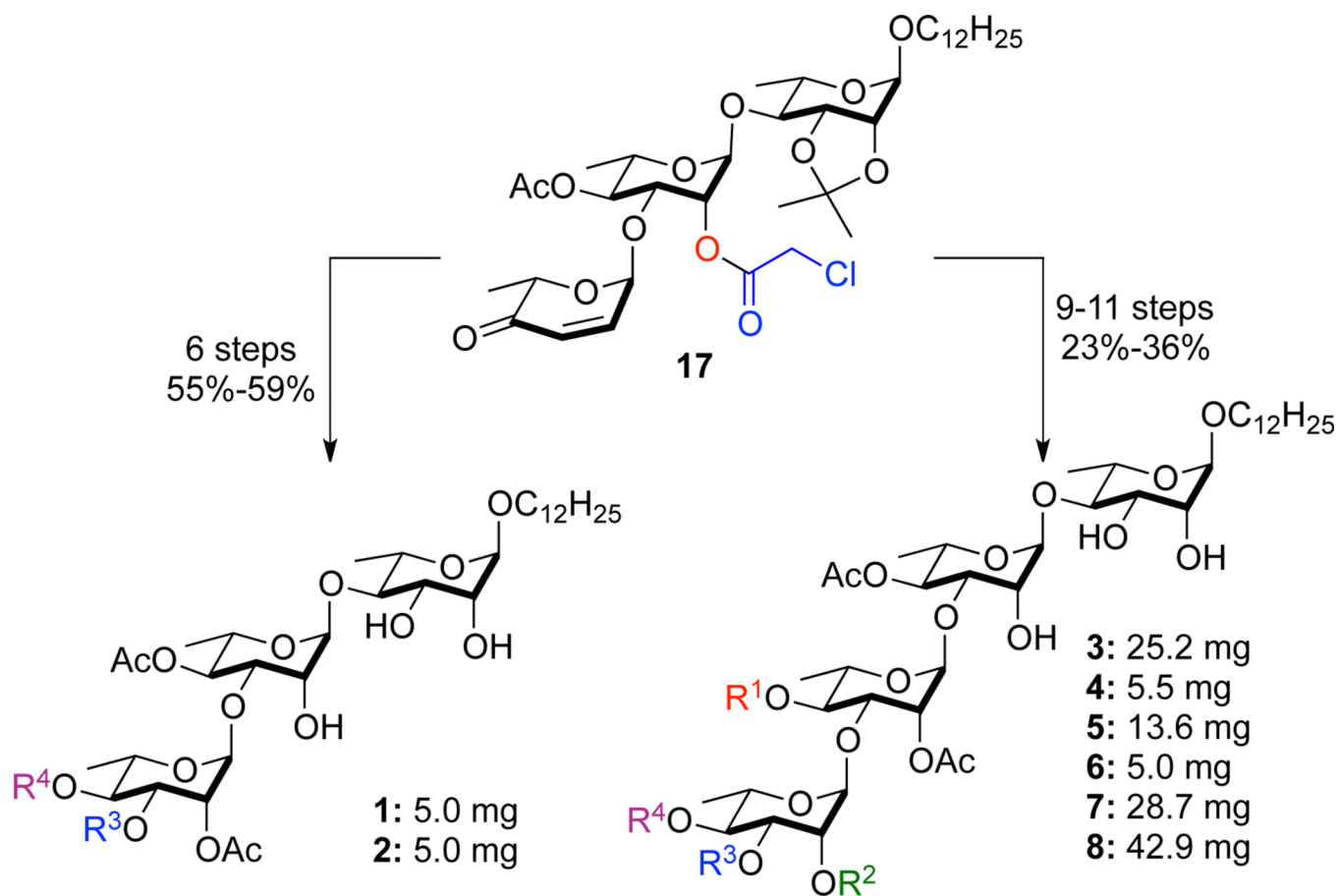
Scheme 3.
Divergent approach to the cleistriosides and cleistetrosides 1–8
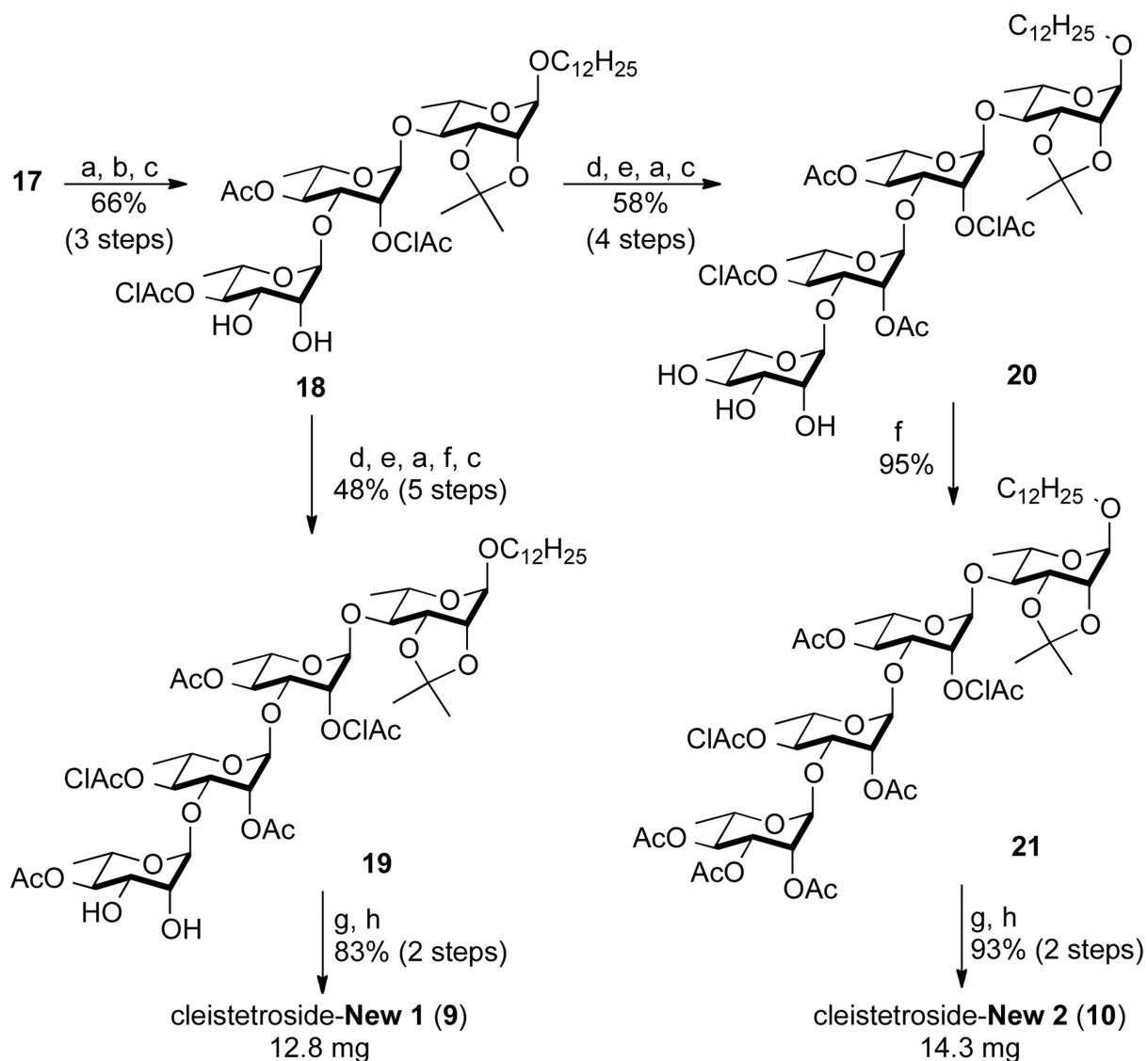
Reagents and conditions: (a) NaBH$_4$, CeCl$_3$, CH$_2$Cl$_2$/MeOH, -78 °C, 2 h; (b) (ClAc)$_2$O, DMAP, Py, rt, 3 h; (c) OsO$_4$, NMO, *t*-BuOH/acetone, rt, 12 h; (d) CH$_3$C(OMe)$_3$, *p*-TsOH, CH$_2$Cl$_2$, 0 °C, 20 min, then AcOH$_{(aq)}$, 20 min; (e) **12**, 5 mol% **Pd(PPh$_3$)$_2$,** CH$_2$Cl$_2$, 0 °C, 2 h; (f) Ac$_2$O, DMAP, Py, rt, 3 h; (g) (NH$_2$)$_2$CS, NaHCO$_3$, Bu$_4$NI, THF, 55 °C, 2 h; (h) 80% AcOH 80 °C, 2 h. **Pd(PPh$_3$)$_2$** = Pd$_2$(dba)$_3$•CHCl$_3$/4PPh$_3$

Scheme 4.
Approach to the cleistetroside 9 and 10

Table 1

Antibacterial activity of cleistroside-5 (1) and cleistetroside-2 (3)[3]

Organism	MIC (µg/mL)	
	1	**3**
Staphylococcus aureus (oxacillin-resistant) ATCC 33591	2	2–4
Coagulase-negative staphylococci	2–4	>32
Enterococcus faecalis	2	2–4
Bacillus spp.	2	2
Streptococcus pneumoniae	4–16	4
Corynebacterium spp.	8	16
Viridians group Streptococci	4	4

Table 2

MIC values for cleistrio/cleistetroside derivatives

	R¹	R²	R³	R⁴	MIC (μM)		
					E. coli		*B. subtilis*
					MG1655	*imp*	JH642
1	-	-	H	Ac	>64	>64	32
2	-	-	Ac	H	>64	32	8
3	Ac	H	H	Ac	>64	>64	4
4	Ac	Ac	H	H	>64	>64	8
5	Ac	H	H	H	>64	>64	8
6	H	H	H	H	>64	16	8
7	Ac	Ac	H	Ac	>64	>64	4
8	Ac	Ac	Ac	Ac	>64	>64	>64
9	H	H	H	Ac	>64	16	8
10	H	Ac	Ac	Ac	>64	>64	>64

Antibacterial activity was assessed by MIC method, which was performed by the broth dilution method described by the National Committee for Clinical Laboratory Standard Methods (M7-A6, 2003) (See supporting information).

Table 3

Cancer cytotoxicity of the ten oligosaccharides on NCI-H460 Human Lung Cancer Epithelial Cells

compd	$IC_{50}(\mu M)$	compd	$IC_{50}(\mu M)$
1	90.9	**6**	15.4
2	12.5	**7**	7.5
3	9.1	**8**	16.4
4	12.8	**9**	16.5
5	12.2	**10**	9.8

The results are an average of at least three independent experiments.

Table 4

Growth inhibition GI50 evaluation of cleistrioside and cleistetroside analogues (μM)

Cell type	Cell line	1	2	3	4	7	8	10
Leukemia	RPMI-8226	4.12	1.00	1.86	2.84	0.96	1.04	0.92
	SR	3.20	1.20	1.45	3.83	0.86	0.92	0.85
Non-Small Cell	A549	6.69	2.08	9.47	8.18	1.18	0.80	0.97
Lung Cancer	NCI-H460	6.32	1.54	7.58	8.43	0.94	0.93	0.96
Colon Cancer	HCT-15	8.39	7.38	7.54	8.33	1.01	0.82	1.52
	HT-29	4.35	1.21	8.02	8.13	0.97	0.91	0.91
CNS Cancer	SF-539	7.11	3.10	4.70	7.17	0.83	0.80	0.91
	SNB-75	3.42	0.90	1.36	2.18	0.50	0.54	0.62
Melanoma	MDA-MB-435	7.68	1.49	6.00	7.25	0.83	0.88	0.84
	SK-MEL-5	6.21	1.23	1.76	6.27	0.74	0.76	0.82
Ovarian Cancer	OVCAR-3	5.91	1.49	4.84	6.07	0.77	0.84	0.80
	ADR-R	9.29	7.92	9.15	8.90	3.38	0.92	2.24
Renal Cancer	A498	6.73	5.13	7.57	5.64	0.73	0.67	0.02
	CAKI-1	8.78	6.57	8.45	9.24	1.42	0.86	0.60
Prostate Cancer	PC-3	2.37	0.89	5.30	6.55	0.82	0.71	0.75
	DU-145	7.94	2.48	7.92	7.47	0.80	0.78	0.79
Breast Cancer	MCF7	4.84	1.62	1.74	5.29	0.87	0.89	0.94
	BT-549	7.90	4.54	8.46	8.39	0.94	1.05	0.88

Growth inhibition of 50% (GI50) is calculated from [(Ti-Tz)/(C-Tz)] × 100 = 50, which is the compound concentration resulting in a 50% reduction in the net protein increase (as measured by SRB staining) in control cells during the compound incubation. Compounds **5**, **6** and **9** were excluded because they did not pass the initial signal dose screen (10 μM).