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Asymmetric Total Synthesis Enables Discovery of Antibacterial Activity of Siladenoserinols A and H

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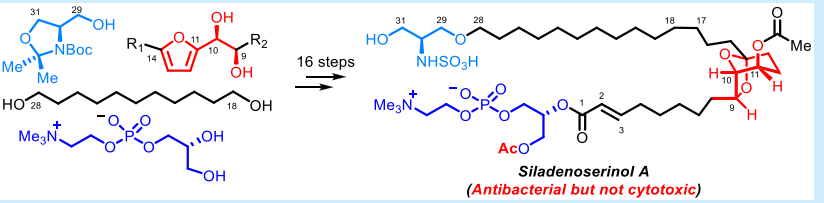
Supporting Information



ABSTRACT: Siladenoserinols A and H were found to show moderate inhibitory activity toward p53-Hdm2 interactions. Our total synthesis allowed us to further examine their bioactivities, which revealed that (i) siladenoserinols A and H were not cytotoxic against cancer cell lines and (ii) siladenoserinol A and its desulfamate analogue exhibited significant antibacterial activity against Gram-positive bacteria including MRSA. Our studies demonstrate that siladenoserinols are a promising new class of bactericidal Gram-positive antibiotics without hemolytic activity.

Siladenoserinols A (**1**) and H (**2**) along with 10 congeners were isolated in 2013 from a tunicate of the Didemnidae family and found to show inhibitory activity against p53-Hdm2 interactions (IC_{50} values of 2.0 and 2.5 μM).[1] They are structurally very unique natural products because they contain a medicinally privileged 6,8-dioxabicyclo[3.2.1]octane (6,8-DOBCO) core,[2] serinol sulfamate, and a betaine α-glycerophosphocholine (α-GPC) through 8- and 14-carbons linkages, respectively. In 2018, Doi[3] and co-workers documented the first total synthesis of siladenoserinol A and found that the inhibitory activity of **1** against p53-Hdm2 interaction was much lower (IC_{50} = 17 μM) than originally reported. Our interest in siladenoserinols was prompted by possible construction of the 6,8-DOBCO core with our previously established strategy[4] and, most importantly, by our hypothesis that siladenoserinol A might exhibit antibacterial activity due to the likely interaction of the betaine α-GPC and hydrophilic serinol sulfamate with the polar, negatively charged cell wall of bacteria.[5] Herein, we describe the asymmetric total synthesis and verification of antibacterial activity of siladenoserinol A and its analogues (Figure 1). Siladenoserinol H was also synthesized for the first time through a rather unexpected late-stage ester 1,2-migration, which greatly expanded the utility of our strategy for the synthesis of other members of siladenoserinols.

As illustrated in Scheme 1, our synthetic strategy featured a convergent assembling of three functional sectors: serinol derivative **3**, 6,8-DOBCO **5**, and α-GPC **6a**, through Williamson etherification (**3** + **4**), Julia−Kocienski olefination (**4** + **5**),[6] and Horner−Wadsworth−Emmons (HWE) olefination (**5** + **6**). The 6,8-DOBCO **5** could be synthesized by our previously established Achmatowicz rearrangement/bicycloketalization[7] of furfuryl diol **8**. The preparation of α-GPC **6a** could be achieved by direct regioselective silylation (triisopropylsilyl, TIPS) of commercially available α-GPC (**7**) followed by acylation.

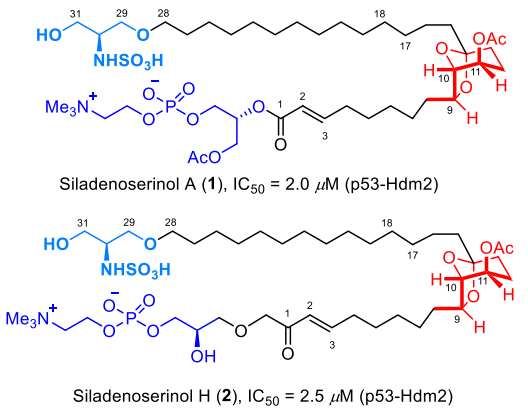


Figure 1. Molecular structures of siladenoserinols A and H.

As depicted in Scheme 2, our synthetic study began with the preparation of furfuryl diol **8** in 85% yield through HWE olefination of furfuryl aldehyde **9** with phenyltetrazole sulfone and Sharpless asymmetric dihydroxylation.[8] Using our green

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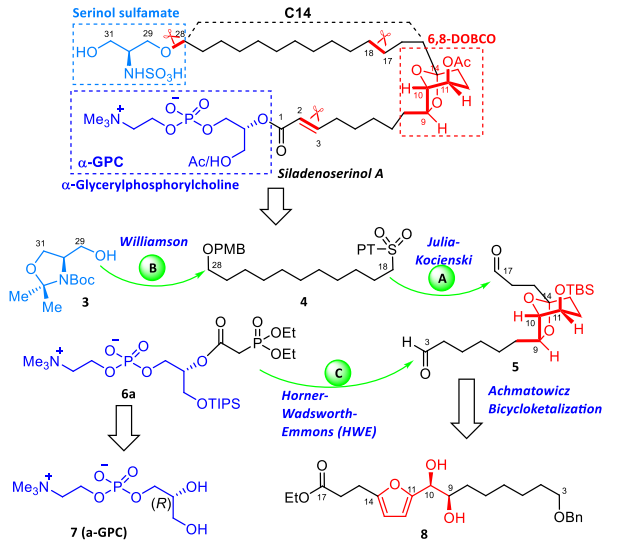
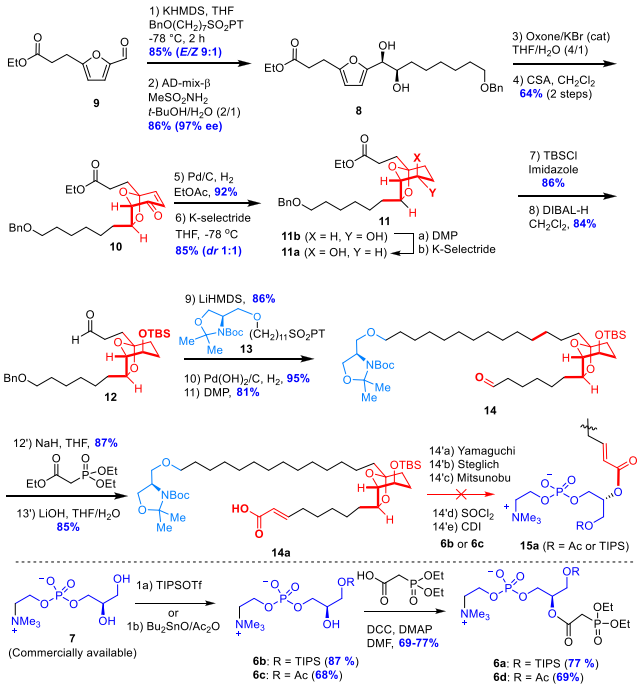
Scheme 1. Retrosynthetic Analysis of Siladenoserinol A



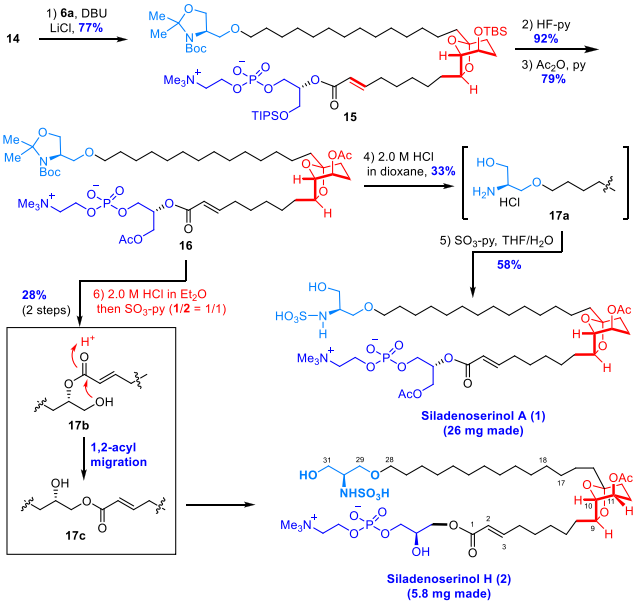
Scheme 2. Synthesis of 6,8-DOBCO Framework 14



Oxone–KBr protocol,[9] Achmatowicz rearrangement of **8** proceeded efficiently and the crude product cyclized in the presence of CSA to provide 6,8-DOBCO **10** in 64% yield. Hydrogenation of the double bond of **10** under palladium catalysis followed by K-Selectride reduction produced a 1:1 diastereomeric mixture of alcohol **11**.

The undesired one (**11b**) could be partially recovered by DMP oxidation and then reduction with K-Selectride to deliver a 1:1 mixture of **11a** and **11b**. It was noted that our six-step synthesis of 6,8-DOBCO **11b** was highly efficient as compared to the 15-step preparation of related 6,8-DOBCO through Au-catalyzed double hydrohydroxylation by Doi.[3] TBS protection of the hydroxyl group of **11a** and DIBAL-H reduction of the ester afforded the required aldehyde for Julia–Kocienski olefination of serinol-derived phenyltetrazole sulfone **13**. Removal of the benzyl protecting group and saturation of the double bond on the side chain was simultaneously achieved by palladium-catalyzed hydrogenation. Dess–Martin periodinane (DMP) oxidation of the resulting alcohol delivered the key aldehyde **14** for HWE olefination with α-GPC **6a** (**14** → **15**, Scheme 3), which was identified to be

Scheme 3. Completion of Total Syntheses of Siladenoserinolipids A and H



feasible after extensive experimentation.[10] The practical challenge to install this betaine α-GPC warranted some comments on other attempted methods. First of all, the hygroscopic and water-soluble properties of betaine α-GPC (**7**) added an enormous challenge in the preparation of α-GPC derivatives (**6a**–**6d**).[11] Second, various classical and well-established esterification methods including Yamaguchi, Steglich, Mitsunobu, $SOCl_2$, and carbonyldiimidazole (CDI) to introduce the known α-GPC **6b** (or **6c**) did not work.[10] Third, HWE olefination of aldehyde **14** with **6d** under Masamune–Roush conditions (note that Doi used it with good yield), resulted in an inferior yield of **15a**.

As depicted in Scheme 3, we found that α-GPC **6a** underwent efficient HWE olefination under Masamune–Roush conditions (LiCl/DBU)[12] to provide the desired product **15** in 77% yield.[13] Next, we removed both TBS and TIPS protecting groups and acetylated the resulting alcohols as diacetate **16**. Removal of acetonide and Boc with 2.0 M HCl in dioxane followed by chemoselective sulfamate formation with SO_3-py furnished siladenoserinol A (21 mg). Notably, our total synthesis required only 16 steps (longest linear sequence), which was considerably shorter than Doi's synthesis (LLS 24 steps). Interestingly, we found that subjection of **16** to 2.0 M HCl in Et_2O (freshly prepared) and then SO_3-py delivered both siladenoserinols A (**1**, 5.7 mg) and H (**2**, 5.8 mg) as a 1:1 mixture, which could be separated by reversed-phase HPLC. The unexpected production of siladenoserinol H was attributed to unanticipated HCl-mediated deacetylation of α-GPC sector of **16** followed by 1,2-acyl migration[14] to the primary position (**17b** → **17c**). The spectroscopic data and optical rotation of our synthetic samples were well consistent with those reported, which suggested that the natural

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Table 1. Cytotoxicity and Antibacterial Activity

	IC_{50} (μM)		MIC (μM)					
	cancer cell lines		Gram-negative bacteria			Gram-positive bacteria		
compd	A549	MGC-803	*E. coli*	mutant *E. coli*[b]	*P. aeruginosa*	*M. luteus*	*B. subtilis*	MRSA
1	100	78	>50	8	>40	8	6	6
2	>100	>100	>50	6	>40	20	20	20
16	>100	>100	>50	>50	>40	>40	>40	>200
17a	65	35	>50	10	>40	8	8	10
18	>100	>100	>50	>50	>40	>40	>40	>200
19	>100	>100	>50	16	>40	20	16	10
C1*[a]	>100	8						
C2*[a]			6	6	>60	40	3	>60
C3*[a]			>40	0.8	>40	1	0.8	0.5

[a]C1*: cisplatin (control). C2*: kanamycin (control). C3*: Vancomycin (control). All bioactivity experiments were performed for three times. [b]Mutant *E. coli* is outer-membrane permeable and Low MIC value suggests the outer-membrane is not target.

siladenoserinols A and H were successfully synthesized in an enantioselective way.

Cytotoxicity and Antibacterial Activity

Since siladenoserinol A was reported to be cytotoxic against cancer cell line A549 (80% cell viability at 10 μM), we first re-examined the cytotoxicity of siladenoserinols A and H, **16** and **17a**, and simplified analogues **18** and **19** (Table 1).[15] To our surprise, none of these compounds were cytotoxic against A549 and MGC-803. Next, we tested our hypothesis that these surfactant-like compounds might possess antibacterial activity as many antibacterial quaternary ammonium compounds.[16] To our excitement, siladenoserinol A and desulfamate analogue **17a** exhibited potent and selective antibacterial activity against Gram-positive bacteria including *Micrococcus luteus* (MIC = 8 μM), *Bacillus subtilis* (MIC = 6 μM) and methicillin-resistant *Staphylococcus aureus* (MRSA, MIC = 6 μM). Intriguingly, siladenoserinol H (**2**) was much less potent, which suggests the ester linkage of at C2′ of the α-GPC sector was beneficial to enhance antibacterial activity. No antibacterial activity was observed for **18**, which suggested that the absence of 6,8-DOBCO (**18**) as compared to siladenoserinol A resulted in a loss of antibacterial activity. In contrast to **17a** and **1**, their precursor **16** did not show any antibacterial activity. These results indicated that the hydrophilic serinol sector and its sulfamate analogue were important to antibacterial activity. We also evaluated the antibacterial activity of these compounds against other Gram-negative bacteria including *Enterobacter cloacae*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, and *Pseudomonas aeruginosa*,[17] and no obvious activity (MIC > 50 μM) was observed (see the Supporting Information). The highly specific activity against Gram-positive bacteria was attributed to their amphiphilic property: these compounds containing betaine α-GPC sector belong to quaternary ammonium compounds (QACs) which have been known as cationic surfactants with potent antimicrobial activity because the cationic ammonium can interact with the negatively charged cell membrane of bacteria, disrupt its integrity, and lead to the leakage of cytoplasmic matters.[18]

Time-Kill Experiments

To further determine whether they were bactericidal or bacteriostatic,[19] we performed time-kill kinetics experiments (Figure 2). We found that all four examined compounds: siladenoserinols A(**1**)/H(**2**) and analogues **17a**/**19**, exerted rapid in vitro bactericidal activity (the colony-forming unit has a 3-log deduction in one hour). Notably, compared with vancomycin, a quick bactericidal effect was observed after bacteria exposure to our synthetic compounds. These results further consolidated the observation of quick bactericidal activity against MRSA biofilm.

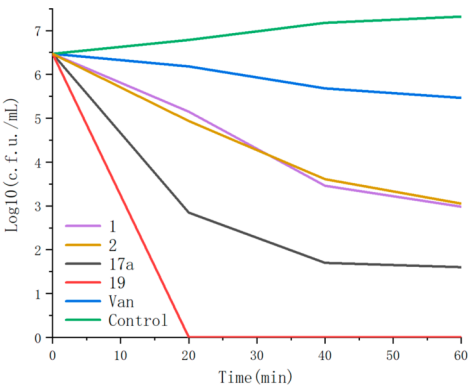

Figure 2. Time-kill experiment. MRSA were grown to early phase (2h) and added ten times the MIC of antibiotics to the culture.

Hemolytic Activity and Analysis of Lysis

Finally, we evaluated hemolytic activity and lysis of siladenoserinols and its analogues with fresh red blood cells isolated from healthy rabbits (Figure 3). Siladenoserinols A and H and **17a** did not show significant hemolytic activity (<5%) at a concentration of >50 μg/mL. Therefore, these promising antibacterial compounds were in the safety range (5%), which holds a great promise for further pharmaceutical development. Our lysis experiments (Figure 3d) indicated that these compounds did not break down the mammalian cell membrane and thus can be considered safe. We should not, however, rule out bacterial membrane cell as the target of these compounds. It would be necessary to perform SYTOX or PI assays in live bacteria in order to determine the membrane targeting.[20] It should be noted that penicillin and related β-lactam antibiotics killed bacteria of defective cell wall through enzymatic lysis.[21] Although many more efforts needed to elucidate the target and the mode of action, we believe siladenoserinols are a new promising class of Gram-positive antibiotics that might combat antibiotic-resistant bacteria (MRSA).

In summary, we have achieved the asymmetric total syntheses of siladenoserinols A and H through a very convergent strategy, which features (i) highly efficient

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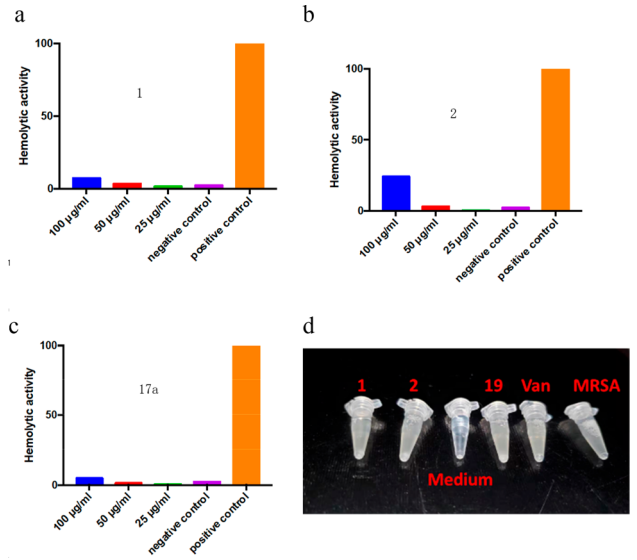

Figure 3. Hemolytic activity in different concentration. (a) Hemolytic activity of siladenoserinol A (**1**), (b) hemolytic activity of siladenoserinol H (**2**), (c) hemolytic activity of compound **17a**, and (d) lysis experiments of **1**, **2**, **19**, and vancomycin (positive control) against MRSA (200 μL of MRSA culture liquid with OD600 value equal to 1.0, then treated with compound at 10 times of the MIC for 24 h. Hemolytic activity was determined with fresh red blood cells isolated from healthy rabbits and used 10% Triton X-100 as a positive control.

enantioselective construction of a 6,8-DOBCO core (six steps) with Achmatowicz rearrangement−bicyclization and (ii) efficient unification of betaine α-glycerylphosphorylcholine derivative and 6,8-DOBCO using robust Horner−Wadsworth−Emmons olefination. Our total synthesis enables us to examine their unknown biological activity, which reveals that siladenoserinol A and its desulfamate analogue **17a** are not cytotoxic against two cancer cell lines but exhibited potent and selective antibacterial activity against Gram-positive bacteria. Time-kill kinetics, hemolysis, and lysis experiments suggest that siladenoserinols and analogues are bactericidal but not lysing the cell membrane. A new mode of action was suspected to operate, and more experiments are needed to fully elucidate the mechanism and target for this new class of antibacterial and antibiofilm compounds.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.orglett.9b03857.

Experimental procedures and analytical data for all new compounds (PDF)

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Notes

The authors declare no competing financial interest.

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