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Total Synthesis and Biological Evaluation of Siladenoserinol A and its Analogues

Masahito Yoshida,[a] Koya Saito, [a] Hikaru Kato, [b] Sachiko Tsukamoto, [b] and Takayuki Doi*[a]

Abstract: The total synthesis of a novel p53–Hdm2 interaction inhibitor siladenoserinol A has been achieved. The $AuCl_3$-catalyzed hydroalkoxylation of an alkynoate derivative smoothly and regioselectively proceeded to afford a bicycloketal in excellent yield. A glycerophosphocholine moiety was successfully introduced by the Horner-Wadsworth-Emmons reaction using an originally developed phosphonoacetate derivative. Finally, removal of the acid labile protecting groups, followed by regioselective sulfamate formation of the serinol moiety afforded the desired siladenoserinol A, and its benzoyl analogue was also successfully synthesized. The biological evaluation showed that the sulfamate is essential for the biological activity, and modification of the acyl group on the bicycloketal can improve the inhibitory activity against the p53–Hdm2 interaction.

Siladenoserinol A (**1**) was isolated from the extract of a tunicate of the family didemnidae collected in Indonesia in 2013, and **1** possesses a 6,8-dioxabicyclo[3.2.1]octane skeleton with two long carbon chains containing unique sulfamated serinol and glycerophosphocholine moieties (Figure 1).[1] A variety of natural products containing the 6,8-dioxabicyclo[3.2.1]octane skeleton have shown to exhibit unique biological activities including pheromonization, cytotoxicity against cancer cells, and inhibition of HIV integrase.[2] Interestingly, siladenoserinol A (**1**) exhibits potent inhibitory activity against the p53–Hdm2 (human murine double minute 2) interaction (IC_{50} = 2.0 μM).[1] The tumor suppressor p53 is essential for the induction of the apoptosis of cancer cells. The upregulation of Hdm2 in the cancer cells is well known to downregulate p53 by a direct interaction that subsequently induces ubiquitination of the p53 and triggers proteasomal degradation. Therefore, the p53–Hdm2 interaction has been a target for drug discovery in cancer therapeutics.[3] Several small molecule inhibitors for the above interaction have been extensively developed; for instance, the *cis*-imidazoline derivative nutlin-3a (**2**) strongly inhibits the p53–Mdm2 interaction (IC_{50} = 0.09 μM) by binding to the p53 binding pockets on the Mdm2.[4] Most of the inhibitors related to nutlin-3a (**2**) act as a mimic of three key hydrophobic residues of p53 (Phe19, Trp23, and Leu26). However, the shape and size of siladenoserinol A (**1**) is different from the small molecule inhibitors, suggesting an alternative binding mechanism for p53-Hdm2 interaction. The total synthesis of siladenoserinol A (**1**) could help developing a library of analogues to elucidate the mode of action. Herein, we report the first total synthesis and biological evaluation of a novel p53–Hdm2 interaction inhibitor called siladenoserinol A and its analogues.

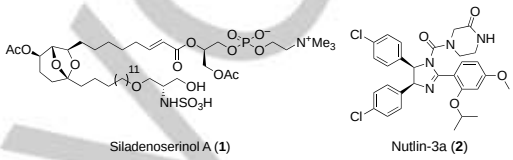
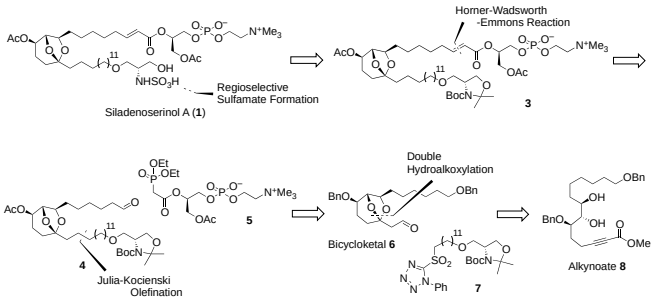


Figure 1. Structures of **1** and **2**.

The retrosynthesis of **1** is illustrated in Scheme 1. The desired **1** could be obtained from **3** by regioselective formation of the sulfamate in a serinol moiety. A glycerophosphocholine moiety in **3** can be introduced by esterification; however, coupling with the corresponding α,β-unsaturated acid is known to be sluggish owing to low reactivity of the acid moiety, and facile isomerization of the resulting α,β-unsaturated ester is also observed.[5] Thus, we chose the Horner–Wadsworth–Emmons (HWE) reaction of **4** with **5** to directly introduce the glycerophosphocholine concomitantly via the formation of an α,β-unsaturated ester. A side chain containing a serinol moiety in **4** can be introduced onto **6** by Julia-Kocienski olefination with **7**, and it can be utilized to prepare analogues possessing the side chain with an arbitrary length. The key intermediate bicycloketal **6** would be regioselectively prepared by double hydroxylation of the alkynoate **8**.[6]



Scheme 1. Retrosynthesis of siladenoserinol A (**1**).

Toward the preparation of the 6,8-dioxabicyclo[3.2.1]octane skeleton,[7] we initially investigated Pd(II)-, Au(I)-, and Au(III)-catalyzed double hydroalkoxylation[8] in a model study using the alkynoate **9**,[9] and the results are summarized in Table 1. Notably, the reaction in the presence of 5 mol% of $AuCl_3$ was smoothly complete within 5 min at ambient

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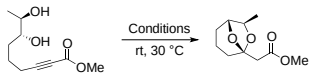
temperature to provide the desired bicycloketal **10** in 79% yield (entry 4).

Table 1. Investigation of the reaction conditions for the double hydroalkoxylation of **9**.

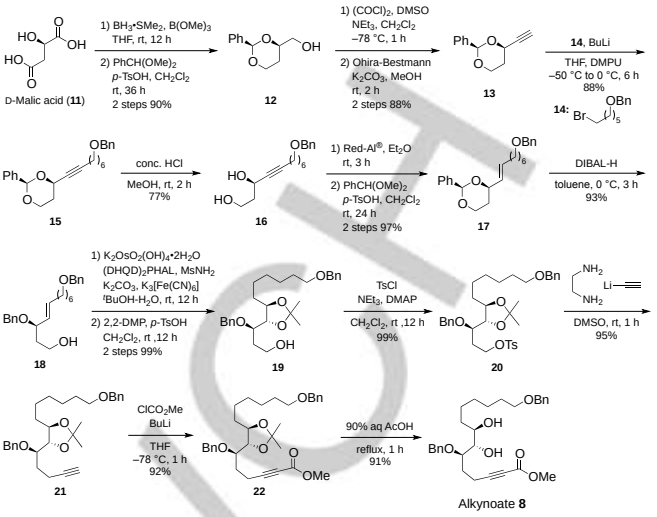


Alkynoate **9** Bicycloketal **10**

Entry	Catalyst [mol%]	Solvent	Time	Yield of **10** [%][a]
1	$PdCl_2(MeCN)_2$ [5]	MeCN	12 h	52
2	$AuClPPh_3/AgOTf$ [5]	MeCN	9 h	69
3	$AuClPPh_3$ [5]	MeCN	10 h	0
4	$AuCl_3$ [5]	MeCN	5 min	79
5	$AuCl_3$ [5]	THF	10 min	56
6	$AuCl_3$ [5]	CH_2Cl_2	15 min	72
7	$AuCl_3$ [5]	Toluene	20 min	75
8	$AuCl_3$ [1]	MeCN	45 min	77
9	AgOTf [5]	MeCN	18 h	0[b]
10	$AlCl_3$ [5]	MeCN	12 h	0[b]

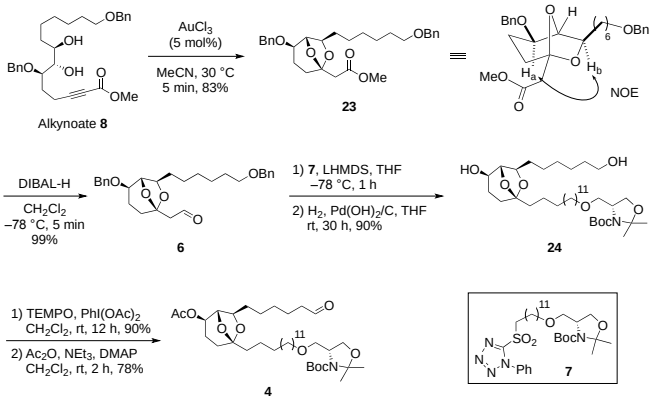
[a] Isolated yield. [b] Substrate **9** was quantitatively recovered.

With the optimal conditions for the bicycloketal formation in hand, the cyclization precursor alkynoate **8** was synthesized and details are shown in Scheme 2. D-Malic acid (**11**) was reduced using BH_3•$SMe_2/B(OMe)_3$, and the resulting 1,3-diol was protected to afford benzylidene acetal **12**. After oxidation of the primary alcohol in **12**, alkynylation of the resulting aldehyde was performed using Ohira-Bestmann reagent[10] to provide alkyne **13** in 88% yield. Alkylation of the terminal alkyne with the bromide **14** afforded **15**, which was treated with HCl/MeOH to yield the diol **16**. Selective reduction of the *endo*-alkyne using Red-Al®, followed by protection of diol afforded **17**. Regioselective cleavage of the acetal moiety in **17** was carried out using DIBAL-H to provide the alcohol **18** in 93% yield. Sharpless asymmetric hydroxylation[11] of the alkene moiety in **18** stereoselectively proceeded, and the resulting 1,2-diol was protected using 2,2-dimethoxypropane under acidic conditions to afford **19**. After modification of the primary alcohol in **19** to the tosylate **20**, alkynylation of **20** using lithium acetylide-ethylendiamine complex furnished the terminal alkyne **21** in 95% yield. Acylation of the terminal alkyne in **21** proceeded smoothly to afford the methyl ester **22**, and removal of the acetal moiety in **22** afforded the alkynoate **8** in 91% yield.



Scheme 2. Preparation of the cyclization precursor alkynoate **8**.

As the precursor alkynoate **8** was successfully prepared, synthesis of the bicycloketal **23** was performed by $AuCl_3$-catalyzed double hydroalkoxylation (Scheme 2). To our delight, the reaction of alkynoate **8** in our optimized conditions afforded bicycloketal **23** in 83% yield as the sole product. The structure of **23** was confirmed by the nOe between Ha and Hb. Methyl ester **23** was reduced by DIBAL-H at –78 °C to give aldehyde **6** in 99% yield. Julia–Kocienski olefination of the aldehyde **6** with sulfone **7**[9], followed by hydrogenation of the resulting alkene concomitantly with removal of the benzyl groups afforded the diol **24** in 90% yield. After selective oxidation of the primary alcohol in **24** by TEMPO-$PhI(OAc)_2$,[12] acetylation of the secondary alcohol on the bicycloketal furnished the aldehyde **4** to be ready for the HWE reaction with the phosphonoacetate **5** (Scheme 3).



Scheme 2. Preparation of the aldehyde **4** via the Au(III)-catalyzed double hydroalkoxylation of **8**.

Because of an unprecedented method for the introduction of a glycerophosphocholine moiety to the aldehyde **4**, the reaction conditions for the HWE reaction with the

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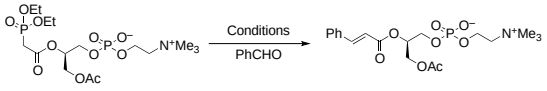
phosphonoacetate **5**[9] were initially investigated using benzaldehyde in a model study and the results are summarized in Table 2. When the reaction using sodium hydride in THF was attempted, the complex mixture was obtained because a base-sensitive phosphonocholine moiety would be lost owing to the strongly basic conditions (entry 1). Regarding the HWE reaction, the Masamune-Roush condition[13] is known to be effective for the base-sensitive substrate to prepare a corresponding α,β-unsaturated ester. As expected, the reaction using reported conditions (DBU, LiCl) was performed at ambient temperature to afford the desired **25** in 52% yield without forming a corresponding *Z*-isomer (entry 2). Notably, the use of LiBr as an additive was more effective in promoting the reaction,[14] and the reaction in THF smoothly proceeded to afford the α,β-unsaturated ester **25** in 67% yield (entry 4).

Table 2. Investigation of the reaction conditions for HWE reaction of benzaldehyde using **5**.

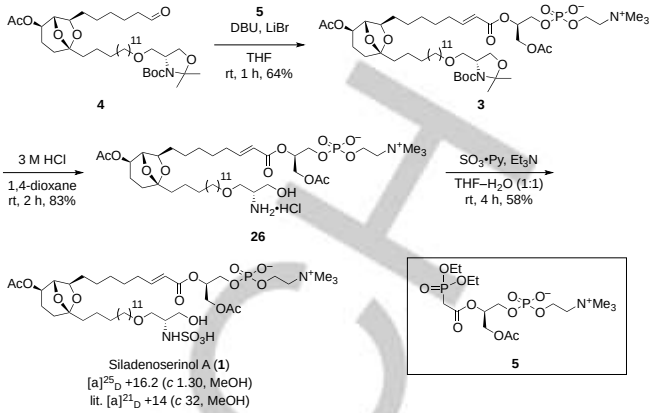


5 **25**

Entry	Base [equiv]	Additive [equiv]	Solvent	Time [h]	Yield of **25** [%][a]
1	NaH [2]	-	MeCN	2	trace
2	DBU [2]	LiCl [2]	MeCN	5	52
3	DBU [2]	LiBr [2]	MeCN	2	61
4	DBU [5]	LiBr [2]	THF	2	67
5	DIEA [5]	LiBr [2]	THF	24	49

[a] Isolated yield.

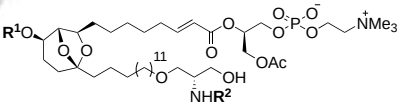
Having an efficient method to introduce a glycerophosphocholine moiety by the HWE reaction, we applied this method for the total synthesis of **1**. The introduction of a glycerophosphocholine moiety to aldehyde **4** was smoothly achieved by treatment with the phosphonoacetate **5** under the optimized conditions (Table 2, entry 4), and the resulting **3** was afforded in 64% yield without losing a base sensitive choline moiety.[15] Finally, removal of the acid-labile protecting groups in **3** quantitatively afforded **26**, and regioselective sulfamate formation of the serinol moiety using the sulfur trioxide-pyridine complex (SO_3•Py)[16] furnished siladenoserinol A (**1**), spectral data of which–including specific rotation–were in good agreement with those of the natural product (Scheme 3).[1]



Scheme 3. Total synthesis of siladenoserinol A (**1**).

After the total synthesis of **1**, the synthesis and biological evaluation of siladenoserinol analogues were then investigated.[17] The results of biological evaluations for the natural product **1** and its analogues are shown in Table 3.[18] The inhibitory activity of synthetic **1** was identical to that of the natural product as a positive control (IC_{50} = 17 μM, entry 1), whereas the desulfamated analogue **26** did not exhibit potent inhibition of p53-Hdm2 (entry 2). Notably, the benzoyl analogue **27** exhibited a fivefold potent inhibitory activity (IC_{50} = 3 μM, entry 3) compared with the natural product **1**. The above observation indicates that the sulfamate moiety is crucial for inhibiting the p53-Hdm2 interaction, and modification of the acyl group on the 6,8-dioxabicyclo[3.2.1]octane skeleton would be a promising derivatization to enhance the inhibition of the p53-Hdm2 interaction.

Table 3. Inhibitory activity for p53–Hdm2 interaction of **1** and its analogues.



Entry	Compound	R^1	R^2	IC_{50} [μM]
1	Siladenoserinol A (**1**)	Ac	SO_3H	Synthetic: 17 (Natural: 17)
2	Desulfamate analogue **26**	Ac	H	-
3	Benzoate analogue **27**	Bz	SO_3H	3

In conclusion, we have achieved the total synthesis of siladenoserinol A (**1**) and its analogues for the first time. The key intermediate bicycloketal **23** was successfully prepared by the $AuCl_3$-catalyzed regioselective double hydroalkoxylation of an alkynoate derivative **8** in 83% yield. As the dioxabicyclo[3.2.1]octane skeleton is often found in the structure of biologically active natural products, thus the $AuCl_3$-catalyzed reaction we developed could be broadly applicable in the preparation of a variety of privileged 6,8-

dioxabicyclo[3.2.1]octane skeletons. The two aliphatic side chains were smoothly installed onto the 6,8-dioxabicyclo[3.2.1]octane skeleton. The phosphonoacetate derivative **5** originally prepared smoothly reacted with the aldehyde **4** under the optimized conditions (DBU, LiBr, THF) to introduce the side chain possessing the glycerophosphonocholine moiety. Finally, removal of the acid labile protecting groups, which was followed by regioselective sulfamate formation furnished the desired **1**. Its benzoyl analogue **27** was also successfully synthesized, and it exhibited a fivefold potent p53–Hdm2 inhibitory activity compared with the natural product. Notably, the potent activity was not observed for its desulfamated analogue, indicating that a sulfamate on the serinol moiety should be crucial for the desired p53-Hdm2 inhibition. Further investigation based on the analogue synthesis would assist in the elucidation for the mode of action and in the discovery of novel drug candidates targeted at the p53–Hdm2 interaction.

Acknowledgements

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Keywords: bicycloketal • glycerophosphocholine • serinol lipid • PPIs • total synthesis

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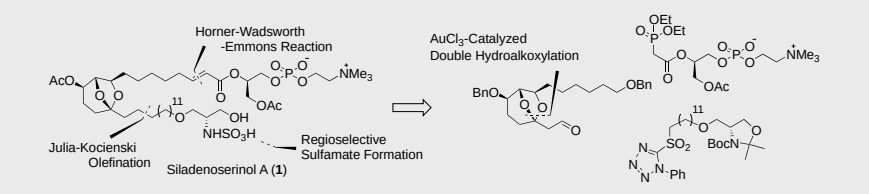

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