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Design, Synthesis, and Biological Activity of Isosyringolin A

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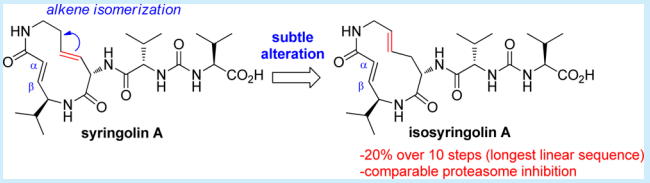
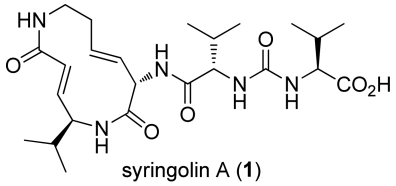
Supporting Information

ABSTRACT: Isosyringolin A, which is an isomer of the proteasome-inhibiting natural product syringolin A, was designed and synthesized to develop analogues that are step economical and synthetically accessible in a practical manner. It was revealed that isosyringolin A exhibited proteasome-inhibitory activity comparable to that of syringolin A and that its derivatization leads to great enhancement in its proteasome inhibitory activity as well as its cytotoxicity against human myeloma cells.



Natural products are a rich resource for drug development.[1] When designing analogues, it is important to design target molecules with comparable or superior biological activity and to consider synthetic step economy to synthesize target molecules.[2] It is often the case that subtle alterations of an atom and a bond connectivity lead to the ready accessibility of natural product analogues.[3] If the analogues maintain the biological activity of the original natural products, designing such analogues by making subtle alterations to the natural product's chemical structures is a strategy that represents a synthetic innovation within the medicinal chemistry community.[4]

Syringolins A and B (**1**, **2**, Figure 1) are naturally occurring 12-membered macrolactams that were isolated from a strain of the plant pathogen *Pseudomonas syringae* pv *syringae* in 1998.[5] They irreversibly inhibit proteasomes by the 1,4-addition of the hydroxyl group of the *N*-terminal threonine (Thr) residue on the β5 subunit to the α,β-unsaturated carboxamide moiety that is embedded in **1**.[6] The inhibition of the proteasome results in an accumulation of unnecessary proteins and ultimately causes cell death.[7] Proteasome inhibitors bortezomib (Velcade) and carfilzomib (Kyprolis) have been approved by the U.S. Food and Drug Administration (FDA) for the treatment of multiple myeloma. Because the mode of inhibition that is used by **1** is different from that used by bortezomib and carfilzomib, **1** is expected to be a new lead for an antimultiple myeloma drug.[8] Syringolin A possesses stronger proteasome-inhibitory activity than **2**. Presumably, the 12-membered macrocycle of **1** is highly strained by virtue of its two alkenes. Upon 1,4-addition of the Thr residue, this strain is released, which provides a driving force to accelerate the reaction rate of **1** with the proteasome, resulting in stronger inhibition of **1** than **2**. These promising biological properties, as well as its characteristic chemical structure, have motivated several groups to synthesize **1**.[9−16] A key to the synthesis of **1** is the construction of the highly strained 12-membered macrocycle. To develop analogues with comparable proteasome-inhibitory activities that are step economical and accessed in a practical, synthetically accessible manner, we designed isosyringolin A (**3**, Figure 2), where one of the C−C double bonds of **1** is isomerized. Molecular modeling of **3** does not suggest that the double-bond transposition causes a significant conformational change of the α,β-unsaturated carboxamide moiety, which is relevant for it to covalently link to the proteasome. From a synthetic point of view, the alkene isomerization results in an allylcarboxamide functionality, which can retrosynthetically be disconnected to the corresponding *N*-acylsulfonamide **4** and allyl alcohol **5**.



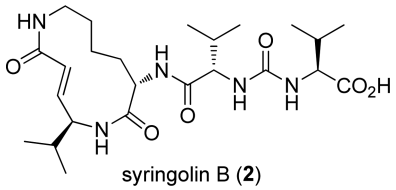


Figure 1. Structure of syringolin A and B.

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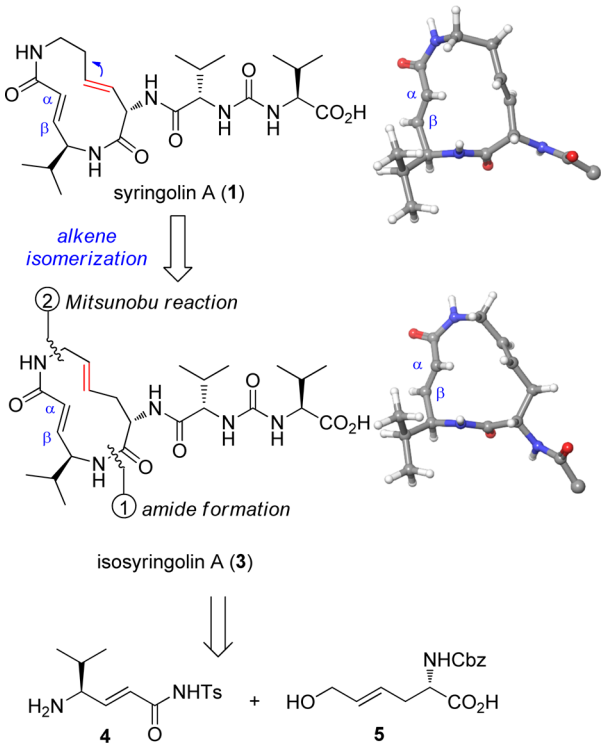
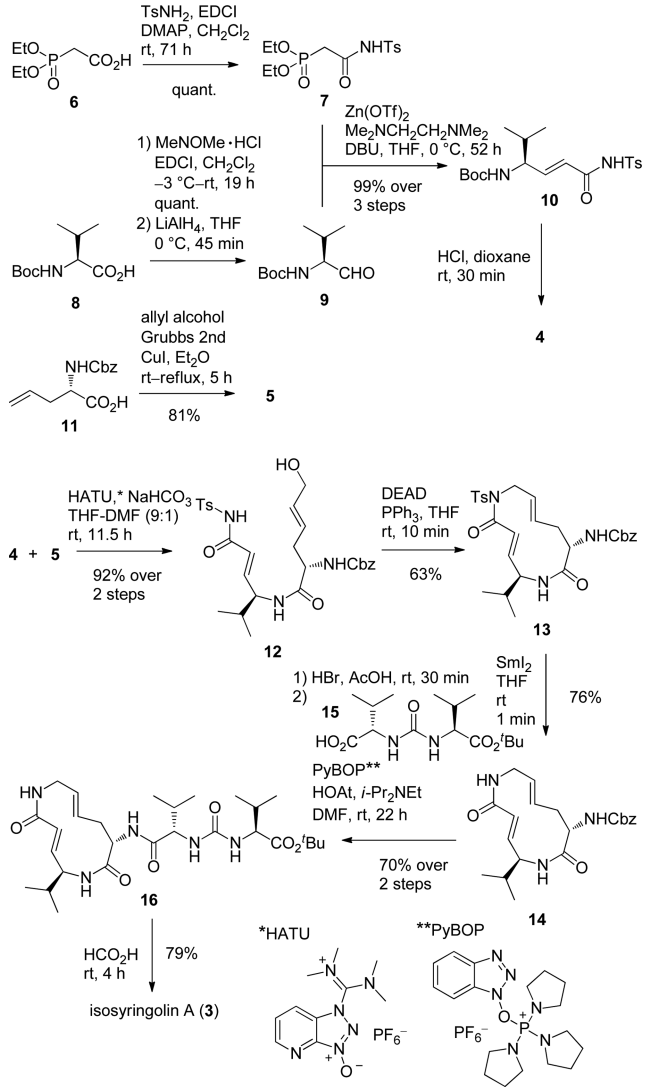


Figure 2. Molecular design of isosyringolin A (**3**).

These two fragments can be connected by a reliable amide-forming reaction, as well as by a Mitsunobu reaction. Herein, we describe the synthesis and biological evaluation of **3** with a side-by-side comparison with **1** and **2**.

The synthesis of **3** is illustrated in Scheme 1. First, fragment **4** was prepared from commercially available diethyl phosphonoacetic acid (**6**) and Boc-L-Val (**8**). Compound **6** was condensed with $TsNH_2$ to give acyltosylamide **7** (EDCI, DMAP, CH_2Cl_2, quant.). Boc-L-Val (**8**) was converted to the corresponding Weinreb amide (MeNHOMe HCl salt, EDCI, HOBt, *N*-methylmorpholine, CH_2Cl_2), which was reduced by $LiAlH_4$ to give the aldehyde **9**.[17] A modified Horner–Wadsworth–Emmons reaction[18] of **9** with **7** ($Zn(OTf)_2$, *N*,*N*,*N*′,*N*′-tetramethylethylenediamine, DBU, THF) to construct the α,β-unsaturated carboxamide gave the fragment **10** in 99% yield over three steps from **8**. Deprotection of the Boc group in **10** (HCl, dioxane) gave the amine **4**. The fragment **5** was prepared from commercially available L-allylglycine (**11**) by cross-metathesis with allyl alcohol, which was catalyzed by Grubbs' second-generation catalyst (CuI, Et_2O, reflux, 81%).[19] The two fragments were condensed by using HATU and $NaHCO_3$ in THF–DMF to provide **12**, the precursor to the cyclization, in 92% yield over the two steps from **10**. The choice of THF as the solvent was critical to improve the yield of **12**; the reaction in DMF resulted in a low yield of **12** (34% yield). The cyclization of **12** by a Mitsunobu reaction proceeded smoothly and within 10 min to afford the desired 12-membered macrocycle **13** in 63% yield (diethyl azodicarboxylate, PPh_3, THF). A cyclization precursor that did not bear the tosyl group at the nitrogen of the α,β-unsaturated carboxamide was also investigated for the cyclization. However, no reaction occurred because greater acidity of the carboxamide moiety is necessary for promoting the cyclization. The cyclization of **12** proceeded well, whereas the macrocyclization in the syringolin A total synthesis proceeded relatively poorly[9,10,12] except for that by

Scheme 1. Synthesis of Isosyringolin A (3)



the Horner–Wadsworth–Emmons reaction reported by Pirrung et al.[11] Presumably, the differences in efficiency is due to the formation of a less-strained ring as well as a different cyclization site on the ring. The tosyl group of **13** was reductively removed by SmI_2 in THF to give **14** while leaving the α,β-unsaturated carboxamide moiety intact.[20] Deprotection of the Cbz group of **14** (HBr, AcOH) was followed by condensation of the liberated amine with the ureadipeptide carboxylic acid **15**, which gave **16** in 70% yield over the two steps from **14**. Finally, the *tert*-butyl group was removed by using formic acid to complete the synthesis of isosyringolin A (**3**) in 79% yield. The subtle alteration of the chemical structure of **1** significantly improved the efficiency with which **3** was synthesized. The total number of steps required for the synthesis of **3** from commercially available materials is 12 (longest linear sequence: 10 steps) with an overall chemical yield of 20%. This is the most efficient synthetic route among the reported total syntheses of **1** and **2**, as well as their analogues.[9−12]

With **3** in hand, its biological activity was evaluated (Table 1). Isosyringolin A (**3**) exhibited a potent $\beta5$ proteasome inhibitory activity with a K_i value of 590 nM, which is 3.5-fold weaker than that of **1** (K_i 170 nM) and 2-fold stronger than

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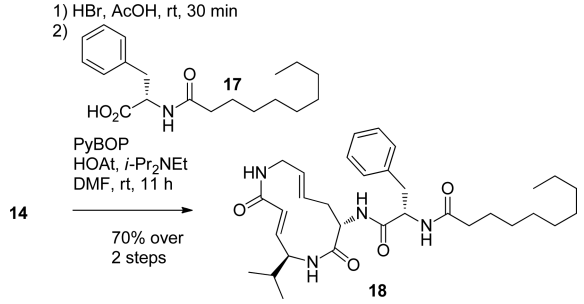
Table 1. β5 (Chymotrypsin-like) Proteasome Inhibitory Activity

	1	2	3	18
K_i (nM)	170	1190	590	1.53

that of **2** (K_i 1190 nM). The potency of **3** was between that of **1** and **2**. These results indicate the importance of including the alkene moiety in the molecular design of **3**.

We have found that the chemical transformation of the ureadipeptide moiety of **1** into the *N*-decanoyl L-phenylalanyl group greatly enhanced its β5 proteasome inhibitory activity as well as its cytotoxicity against human cancer cells.[12] Compound **3** was converted into the corresponding analogue **18** by condensation of **14** with *N*-decanoyl-L-phenylalanine (**17**) as is shown in Scheme 2.

Scheme 2. Synthesis of Isosyringolin A Analogue 18



The analogue **18** exhibited strong β5 proteasome inhibitory activity with a K_i value of 1.53 nM (Table 1). The cytotoxicity of **18** was also investigated; **18** showed a strong cytotoxicity against human myeloma OPM-2 cells with an IC_{50} value of 6.7 nM (Table 2). This potency is comparable to that of

Table 2. Cytotoxicity against Human Myeloma Cells

	IC_{50} (nM)	
cell lines	bortezomib	18
OPM-2	2.8	6.7
bortezomib resistant OPM-2	146	60

bortezomib (IC_{50} = 2.8 nM), which is currently used as a proteasome-inhibitor drug for multiple myeloma. Of significance is its potency against bortezomib resistant OPM-2 cells,[21] against which bortezomib is less effective and has an IC_{50} value of 146 nM and a 52-fold reduction in potency. Importantly, **18** showed potent cytotoxicity against the bortezomib resistant OPM-2 cells (IC_{50} = 60 nM) with only a 9-fold reduction in its activity.

In conclusion, isosyringolin A, which is an isomer of the proteasome-inhibitory natural product syringolin A, was designed and synthesized to develop an analogue with comparable proteasome-inhibitory activity that is step economical and accessed in a practical, synthetically accessible manner. Isosyringolin A (**3**) exhibited potent β5 proteasome-inhibitory activity that was comparable to that of the natural products. The analogue **18** exhibited strong β5 proteasome inhibitory activity as well as cytotoxicity against not only bortezomib-susceptible but also against bortezomib-resistant human myeloma cells. The analogue **18** is readily accessible in 11 synthetic steps (longest linear sequence: 9 steps) from commercially available materials, with an overall chemical yield of 25%. Further optimization would lead to the development of a next-generation proteasome inhibitor that is effective against bortezomib-resistant multiple myeloma. This study suggests new, efficacious molecules may be designed by subtly altering the chemical structures of natural products, which is an approach that represents a significant synthetic innovation within the medicinal chemistry field.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.6b01053.

Complete experimental procedure and characterization data of all the new compounds, β5 proteasome inhibition assay and cytotoxicity assay (PDF)

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Notes

The authors declare no competing financial interest.

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