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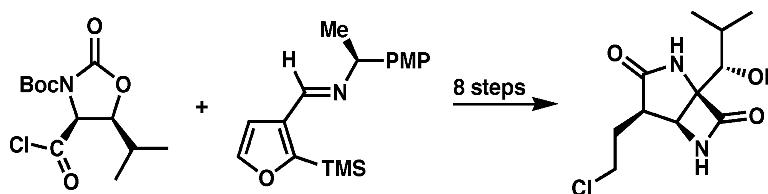

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Proteasome Inhibition by a Totally Synthetic β-Lactam Related to Salinosporamide A and Omuralide

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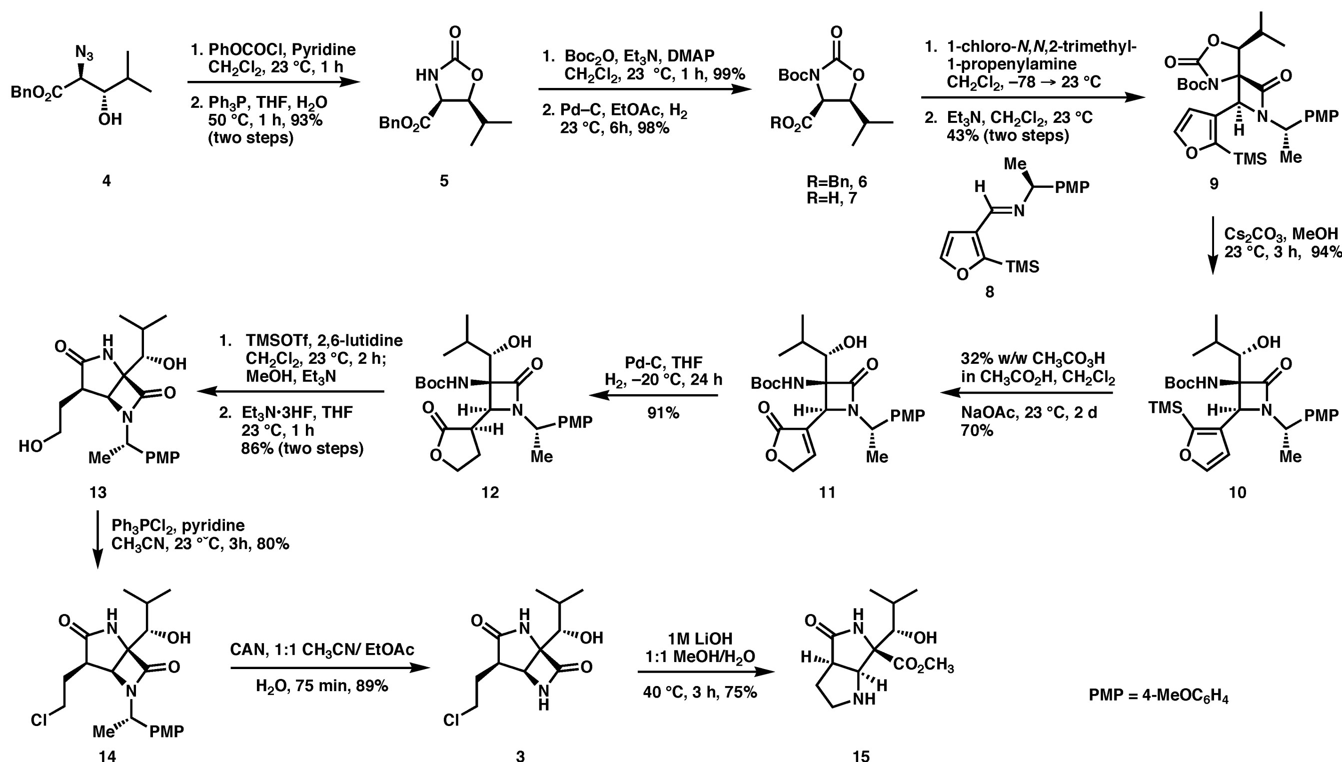
Salinosporamide A (**1**) and omuralide (**2**) are potent naturally derived substances which inhibit proteasome function with very high selectivity.[1−3] Proteasome inhibition offers considerable promise in the therapy of a number of types of cancer and is already used for multiple myeloma.[4] Several routes have been developed for the syntheses of **1** and **2**.[1,5] One potential problem with the use of **1** or **2** as therapeutic agents is their short half-life in solution at pH 7 or in serum (estimated as 5−10 min). Because of this potential shortcoming, we have developed a synthesis of the β-lactam **3**, which is expected to be much more stable than the corresponding β-lactone (or **1** and **2**).

The pathway of the synthesis is outlined in Scheme 1. The known alcohol **4**[6a] was converted to oxazolidinone **5**[6b] (93% yield) by successive acylation with phenyl chloroformate and pyridine in dichloromethane and reduction of the azide function with concomitant cyclization. N-Protection of **5** with *tert*-butylpyrocarbonate−4-(dimethylamino)pyridine (DMAP)−Et_3N afforded **6**. Reductive cleavage of the benzyl ester subunit in **6** provided the carboxylic acid **7**.

Addition of a solution of **7** to a solution of 1-chloro-*N*,*N*-2-trimethylpropenylamine[7] led to smooth formation of the corresponding acid chloride. Rapid addition of the acid chloride to a mixture of the imine **8**[8] and triethylamine gave stereoselectively the β-lactam **9** in 43% yield. Noteworthy in this transformation is the use of an imine derived from (*S*)-(−)-1-(4-methoxyphenyl)-ethylamine. The corresponding imine derived from 4-methoxybenzylamine led to β-lactam but in lower yields (∼12%). Cleavage of the oxazolidinone ring in **9** was accomplished by exposure to Cs_2CO_3−MeOH to afford the alcohol **10** in high yield (94%).[9] Oxidation of the 2-trimethylsilylfuran subunit in **10** by peroxyacetic acid gave the butenolide **11**.[10] Catalytic reduction of **11** provided the butyrolactone **12** in 91% yield, the remainder of the material being the corresponding diastereomer. Cleavage of the *t*-butoxycarboxyl group of **12** was accomplished with trimethylsilyl trifluoromethanesulfonate and 2,6-lutidine in dichloromethane at 23 °C. Any remaining trimethylsilyl trifluoromethanesulfonate was quenched by addition of methanol and triethylamine to the reaction mixture. Fluoride treatment during workup provided the desired butyrolactam **13** in 86% yield. The chlorine atom was introduced by selective reaction of the primary hydroxyl of **13** with dichlorotriphenylphosphorane and pyridine in acetonitrile at 23 °C to give **14** in 80% yield. Oxidative cleavage of the (*S*)-1-(−)-(4-methoxyphenyl)ethylamide protecting group in **14** with ceric ammonium nitrate (CAN) cleanly afforded the β-lactam **3** in 89% yield. We were pleased that **3** is completely stable at pH 7 and 23 °C for 24 h.

Scheme 1



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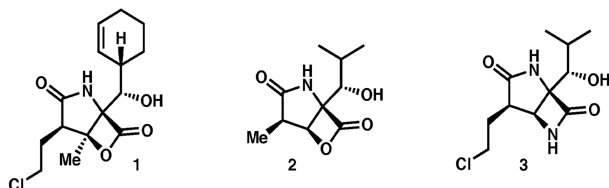

Figure 1.

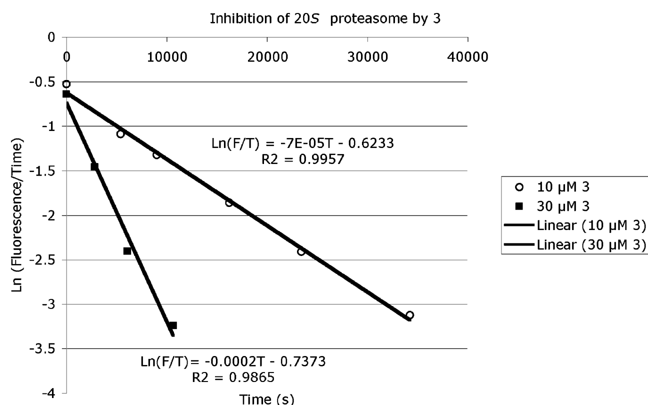

Figure 2. Plot of ln(fluorescence/time) versus time during proteasome inactivation experiments. The slope of these lines are equivalent to k_{obs} for the inactivation of 20*S* proteasome by the β-lactam **3**. The half-lives of inactivation of 20*S* proteasome by **3** are approximately 58 and 165 min at concentrations of **3** at 30 and 10 μM, respectively.

Determination of the inhibitory activity of **3** against the 20*S* proteasome[12] was carried out by a modification of the method reported by McCormack and Dick et al.[11] Incubation of 20*S* proteasome (0.5 μg/mL) with **3** at 10 and 30 μM in pH 7.9 buffer (20 mM Hepes, 0.5 mM EDTA, 0.025% w/v SDS) at 37 °C resulted in a time-dependent loss in the proteasome's ability to hydrolyze Suc-Leu-Leu-Val-Tyr-AMC (50 μM).[12] The progress of proteasome inactivation was determined by addition of Suc-LLVY-AMC to a 2 mL aliquot of the incubation experiment and monitoring the rate of production of free AMC by fluorescence spectrophotometry. A plot of ln(fluorescence/time) versus time provided a linear decay the slope of which gave k_{obs} for proteasome inactivation (Figure 2). The rate of proteasome inactivation was proportional to the concentration of **3** present in the incubation experiments. The 10 and 30 μM time course experiments gave values for k_{obs}/[**3**] as 7 and 6.7 M^{-1} s^{-1}, respectively. To monitor background inactivation of 20*S* proteasome,[13] each of these of experiments was run side by side with a control experiment identical in all respects except for the exclusion of **3**. In the control experiment correlating to 30 μM of **3**, after 3 h, ~30% of initial proteasome activity was lost. In the control experiment correlating to 10 μM of **3**, after 9.5 h, ~50% of the initial proteasome activity remained. In each the corresponding experiments containing **3**, less than 10% of the initial proteasome activity remained at these respective time points. Although the rate of proteasome inhibition by **3** is considerably slower than that for **1** or **2**, the slow rate is more than compensated for by the greatly increased aqueous stability of **3** under physiological conditions.

It seems reasonable that the pathway of proteasome inhibition by the β-lactam **3** follows that of omuralide and salinosporamide A, that is, acylation of a catalytically active threonine of a proteolytic β-subunit. It is likely also that this acylation is rendered irreversible by ring closure involving the chloroethyl group as an electrophile, as appears to be the case for salinosporamide A,[5b] since treatment of **3** with methanolic base afforded the bicyclic pyrrolidine **15**. This fact, the observation of proteasome inhibition in vitro, and the indefinite stability in neutral aqueous solution suggest that **3** is a worthy candidate for further biological evaluation.

Acknowledgment. We thank Drs. Lawrence Dick and Christopher Tsu (Millennium Pharmaceuticals, Inc.) for advice on determining 20*S* proteasome inhibition.

Supporting Information Available: Experimental synthetic procedures as well as physical and spectral data for compounds **3** and **5**−**15**, experimental procedures for incubation of 20*S* proteasome with **3**, and determination of inactivation rates. This material is available free of charge via the Internet at http://pubs.acs.org.

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(12) Obtained from Boston Biochem; Suc = succinyl; AMC = aminomethylcoumarin.
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