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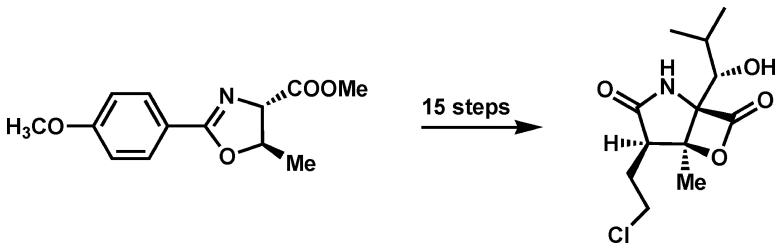

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An Efficient, Stereocontrolled Synthesis of a Potent Omuralide−Salinosporin Hybrid for Selective Proteasome Inhibition

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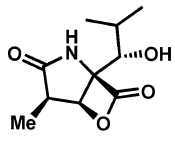
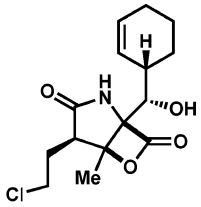
Omuralide (**1**)[1] and the corresponding thiolester with *N*-acetyl-(*S*)-cysteine (lactacystin, a terrestrial microbiol product[2]) have been shown to be remarkably effective and selective inhibitors of proteasomes, the biomolecular machines responsible for the breakdown of polyubiquitin-tagged proteins. For this reason and also because proteasome inhibition is a promising approach to anticancer therapy,[3] there have been numerous studies on the synthesis of omuralide, lactacystin, and analogues leading to a useful structure−activity correlation.[1a] This field has been further advanced by the recent discovery of the marine microbiol product salinosporamide A (**2**), which displays even greater potency than omuralide in vitro in whole cell assays of antiproteasome and antitumor activity.[4] We recently reported the first total synthesis of **2** by a stereocontrolled process that started from (*S*)-threonine and employed an internal Baylis−Hillman reaction to establish the γ-lactam core.[5] We report in this paper a new approach to the synthesis of omuralide−salinosporamide-type proteasome inhibitors as applied specifically to the construction of new hybrid molecules based on the structures **1** and **2**, specifically, in this instance, the β-lactone **3** (Scheme 1). The synthesis of **3** is unusually concise and includes novel methodology for appendage introduction, diastereoselective γ-lactam ring formation, methyl ester cleavage, and β-lactone formation.



1

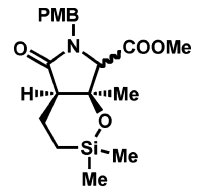


2

The synthesis of the omuralide−salinosporamide hybrid **3**, as outlined in Scheme 1, started with oxazoline **4**, which is readily available from (*S*)-threonine as indicated previously.[5] Reaction of **4** in THF at −78 °C with 1.2 equiv of potassium hexamethyldisilazide in toluene at −78 °C for 1 h provided an enolate which was treated successively with $ZnCl_2$ (1.3 equiv, 10 min, −78 °C) and isobutyraldehyde (1.5 equiv, 8 h, −78 °C) to produce the required aldol product in 95% yield with 10:1 diastereoselectivity. Recrystallization from hot ethyl acetate afforded a major product, mp 118−120 °C (81%), which was converted to the corresponding *tert*-butyldimethylsilyl (TBS) ether **5** in 95% yield. Reductive cleavage of the oxazoline **5**[5] followed by Swern oxidation (at −78 °C with the reagent from Me_2SO and ClCOCOCl in CH_2Cl_2 followed by Et_3N) generated the α-amino ketone **6** in 89% yield. N-Acylation of **6** with acrylyl chloride (3 equiv) and triisobutylamine in CH_3CN solution at 23 °C for 24 h resulted in incomplete reaction, and so additional acrylyl chloride (3 equiv) and tertiary amine (3 equiv) were added on day 2 and day 3. Isolation of the product after day 4 afforded the keto acrylamide **7** (79% yield) as a colorless solid, mp 118 °C. The structure of **7** was confirmed by X-ray crystallographic analysis (see Supporting Information).

Cyclization of the keto acrylamide **7** was next effected stereoselectively and, in the required sense, by an unprecedented application of the Kulinkovich reagent[6] (3.5 equiv) from the reaction of Ti(*i*-PrO)$_4$ (4 equiv) and cyclopentylmagnesium chloride (7 equiv) in *t*-BuOMe at −40 °C for 30 min. The resulting black−brown Ti(II)−cyclopentene complex at −40 °C was treated with the keto amide **7**, and the mixture was maintained at −40 °C for an additional 30 min after which time 5 equiv of I_2 was added to effect iodination of the intermediate α-titanamethyl-γ-lactam (at −40 °C for 2 h and 23 °C for 2 h). Acidification of the reaction mixture with 1 N hydrochloric acid and extraction with methylene chloride afforded an iodomethyl-γ-lactam, which after treatment with Et_3N (30 min at 23 °C) underwent elimination of the iodine substituent β to the γ-lactam carbonyl to give lactam **8** as the only detectable stereoisomer.[7,8] Pure **8** was obtained as a colorless solid, mp 59−63 °C, after flash column chromatography in 85% overall yield from **7**. Silylation of the tertiary hydroxyl group in **8** by $BrCH_2Si(CH_3)_2Cl$ and replacement of bromine by iodine provided the iodo olefin **9**.

The cyclization of the unsaturated iodide **9** (or the corresponding bromide) turned out to be unexpectedly challenging. Under the radical cyclization conditions that had been employed in our original synthesis of salinosporamide A (Bu_3SnH, AIBN, C_6H_6, 80 °C),[5] the required product **10** was formed in only minor amount and the principal products were the two diastereomeric esters (**A**) that correspond to cleavage of the carbon−carbon bond connecting the α-silyloxyisobutyl appendage to the lactam ring. This highly unusual cleavage, which was not observed with the cyclization product **10** under the reaction conditions, appears to be due to a thermal fragmentation of the same α-carbonyl stabilized radical cyclization intermediate that leads to **10** (perhaps driven by steric repulsion between the substituents on the β and γ quaternary carbons). Support for this idea came from cyclization experiments at lower temperatures. Thus, when the cyclization was conducted at −78 °C, after addition of Et_3B to a mixture of **9**, Bu_3SnH, and Et_2AlCl in air-containing toluene, the required product **10** was obtained in 93% yield with essentially complete stereoselectivity.[9]

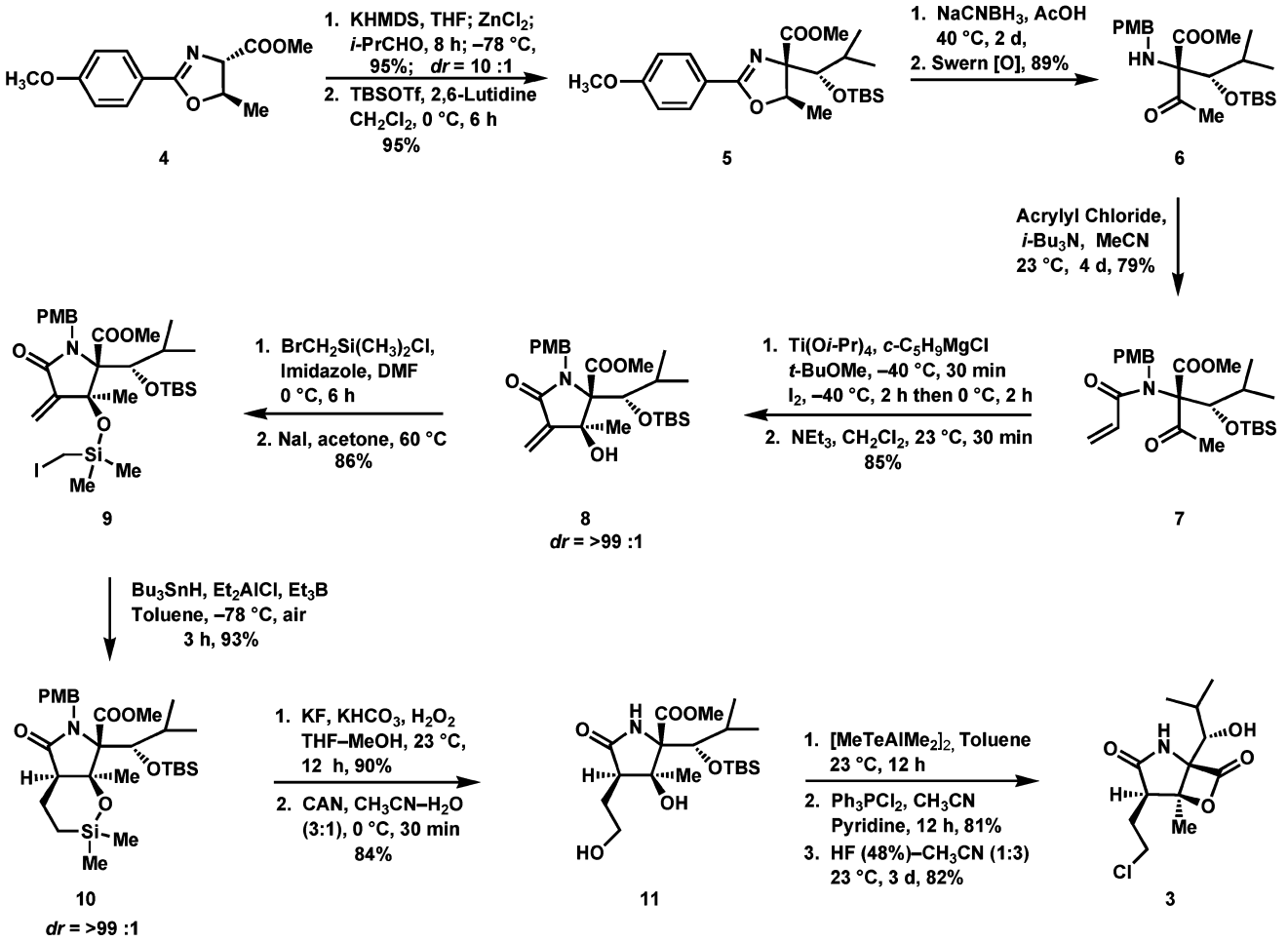


A

Hydroxy desilylation and N-deprotection of **10** followed using the methodology previously described for the synthesis of salinosporamide A[5] to afford the dihydroxy lactam **11**. Attempts to cleave

10.1021/ja052376o CCC: $30.25

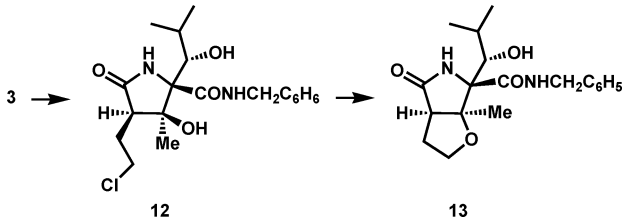
Scheme 1



either the methyl ester or the silyl ether groups of **11** met with complete failure under a wide variety of conditions, clearly because of the great ease of retroaldol cleavage of the same carbon−carbon bond that was involved in the conversion of **9** to the undesired product **A** under radical cyclization conditions. Because every one of available general reagents[10] for the conversion of methyl esters to the corresponding carboxylic acids failed with substrate **11**, a totally new method was sought that could deal with the issues of (a) strong steric screening of the methoxy carbonyl group in **11** and (b) strong driving force for retroaldol cleavage from the tetrahedral alkoxide intermediate that results from nucleophilic addition to the methoxy carbonyl group of **11**. Our attention was directed to a reagent that could effect $COOCH_3$ demethylation by selective attack on the methyl group rather than the ester carbonyl and that could operate under mild conditions in terms of temperature and basicity. Success was realized with a new reagent, $[Me_2AlTeMe]_2$, that is readily generated by heating tellurium powder (1.2 equiv) and trimethylaluminum (1 equiv) in toluene at reflux for 6 h and cooling the product to ambient temperature to give a 0.8 M solution of $[Me_2AlTeMe]_2$ in toluene. Treatment of the dihydroxy ester **11** with a freshly prepared solution of $[Me_2AlTeMe]_2$ (0.8 M in toluene) at 23 °C for 12 h under nitrogen followed by quenching of the reaction mixture with 1 N hydrochloric acid and extractive isolation with ethyl acetate afforded the dihydroxy acid corresponding to **11** cleanly. **CAUTION**: Organotellurium reagents should be used only in a well-ventilated hood; treatment with 1 N hydrochloric acid (or bleach) effects their destruction and deodorization. When the crude acid was subjected to reaction with 4 equiv of Ph_3PCl_2 in dry 1:1 CH_3CN/pyridine at 23 °C for 12 h, it was transformed directly into the TBS ether of **3**, which was obtained as a colorless oil in 81% yield after extractive isolation with ethyl acetate and flash chromatography on silica gel. This very efficient operation combines side chain chlorination with a novel method of β-lactone formation in a single step. Finally, desilylation of the TBS ether of **3** using 3:1 CH_3CN and 48% hydrofluoric acid at 23 °C for 3 days afforded the target omuralide−salinosporamide hybrid **3** as a colorless solid in 82% yield after extractive isolation (EtOAc) and flash chromatography on silica gel. Found for pure **3**: $R_f = 0.45$ (silica gel plate, EtOAc−hexane 1:1); mp 144−145 °C $[\alpha]^{23}_D$ −22.5 (*c* 0.5, $CHCl_3$). FTIR (film) ν_{max}: 3222, 2960, 2944, 2867, 1833, 1710, 1254, 1090, 1059, 852, 825, 777 cm^{-1}. ^1H NMR ($CDCl_3$, 500 MHz): δ 6.43 (1H, (brs)), 3.97 (1H, m), 3. 84 (1H, t, $J = 6.5$ Hz), 3.77 (1H, m), 2.82 (1H, t, $J = 7.5$ Hz), 2.27 (1H, m), 2.12 (1H, m), 1.93 (1H, m), 1.82 (3H, s), 1.12 (3H, d, $J = 7.0$ Hz), 1.08 (3H, d, $J = 7.0$ Hz). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 177.72, 167.47, 85.89, 79.04, 71.94, 44.92, 42.49, 31.53 28.24, 19.92, 19.74, 18.76. HRMS (ESI) calcd for $C_{12}H_{19}ClNO_4$ $[M + H]^+$ 276.1002, found 276.1006.

The biological activity of **3** was evaluated relative to omuralide (**1**) by measurement of the rates of inactivation of the β5-subunit of the human 20*S* proteasome. The potency of **3** by this standard is about 2.5 times that of omuralide (**1**) but somewhat less than that of salinosporamide A (**2**). We anticipate that **3** may have comparable whole cell potency to salinosporamide, although these studies are still in progress. One reason for this expectation is the possibility that the chlorine substituent in **2** and **3** may be critical to ensuring that the covalent attachment of these agents to the proteasome is irreversible. This possibility is suggested by chemical

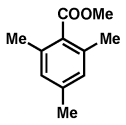
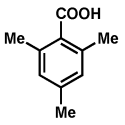
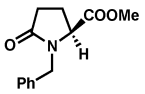
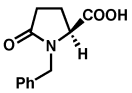
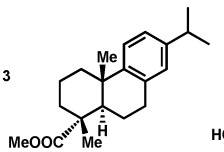
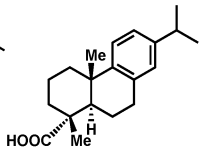
evidence for the facile displacement of chlorine from the α-side chain of **2** or **3** by a free tertiary hydroxyl group at C-β. Thus, reaction of **3** with benzylamine in THF at 23 °C leads to rapid cleavage of the β-lactone ring to form the amide **12** which upon exposure to K_2CO_3 in CH_3OH affords the bicyclic tetrahydrofuran derivative **13**. In this experiment, benzylamine serves as a chemical model for the nucleophilic β5-subunit of the proteasome. To the extent that this model is valid, a simple explanation for the potency of salinosporamide A in cell-function assays may be found in the irreversibility of proteasome inactivation associated with tetrahydrofuran ring closure, as exemplified by **12** → **13**.[11]



The efficient synthesis of antiprotealide[11] (**3**) was made possible by the success of a number of crucial individual steps including: (1) the doubly diastereoselective aldol coupling of a zinc enolate via a preferred cyclic chair-formed transition state; (2) the Kulinkovich reagent-mediated internal carbo−titanation of the acrylamide **7**, which leads to **8** in excellent yield and with very high diastereoselectivity; (3) the striking effectiveness of low-temperature conditions for promoting the diastereoselective cyclization **9** → **10**; and, finally (4) the great success of the novel reagent $[Me_2AlTeMe]_2$ for the demethylation of ester **11**.

Preliminary studies in this laboratory have shown that this mild method for methyl ester cleavage can be used to good advantage with a wide range of hindered esters. Three further examples of the new process are given in Table 1.

Table 1. $[Me_2AlTeMe]_2$-Promoted Conversion of Esters into Carboxylic Acids in Toluene at 23 °C

entry	ester	acid[a]	$[Me_2AlTeMe]_2$ (eq)	time (h)	yield (%)[b]
1			1.2	6	91
2			2.2	6	88
3			2.2	10	88

[a] All products were characterized by ^{1}H NMR, IR, and mass spectroscopy. [b] Isolated yield of pure product.

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Supporting Information Available: Experimental procedures for the steps shown in Scheme 1 are given along with characterization data for each product and the complete list of authors for ref 3a. The complete list of authors for ref 3a. This material is available free of charge via the Internet at http://pubs.acs.org.

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(11) Because of the availability of the hybrid molecule **3** by the synthetic route that is summarized in Scheme 1 and also because of its likely irreversible mode of action in vivo, we assign it the name "antiprotealide" in the expectation that it represents a convenient and useful tool in experimental biology and medicine. For a recent review of natural product hybrids, see: Tietze, L. F.; Bell, H. P.; Chandrasekhar, S. *Angew. Chem., Int. Ed.* **2003**, *42*, 3996−4028.

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