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Enantioselective Total Synthesis of (−)-Salinosporamide A (NPI-0052)

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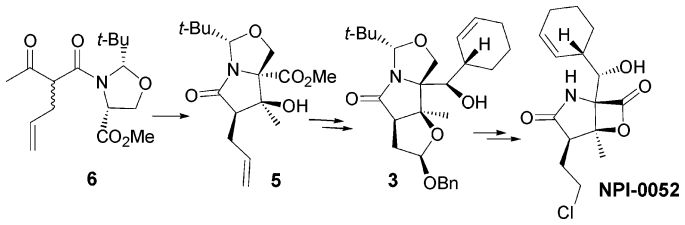
ABSTRACT



A novel enantioselective total synthesis of 20S proteasome inhibitor Salinosporamide A (NPI-0052; 1) is presented. Key features include intramolecular aldol cyclization of 6 to simultaneously generate the three chiral centers of advanced intermediate 5, cyclohexene ring addition using *B*-2-cyclohexen-1-yl-9-BBN, and inversion of the C-5 stereocenter by oxidation followed by enantioselective enzymatic reduction.

Salinosporamide A (NPI-0052; **1**), a secondary metabolite of the marine actinomycete *Salinispora tropica*, is a potent inhibitor of the 20S proteasome that is currently in clinical trials for the treatment of cancer.[1−3] Structurally, **1** comprises a γ-lactam-β-lactone bicyclic ring system substituted with methyl, cyclohex-2-enylcarbinol, and chloroethyl substituents that give rise to specific and mechanistically important interactions within the proteasome active site.[4] Clinical supplies and analogues of **1** have been generated by saline fermentation,[3,5,6] and the results of SAR studies of these and other analogues prepared by semisynthesis have been reported previously.[3,6] However, the number of analogues accessible through semisynthesis is limited by the labile nature of the β-lactone ring. We therefore designed a novel total synthesis of **1** to provide access to a broader suite of analogues. To date, several total syntheses of **1** have been reported,[7−10] with all routes progressing toward an aldehyde intermediate through which the cyclohexene ring is installed via the Corey strategy[7] and converging upon a common sequence of steps.[11] The enantioselective routes employ stepwise introduction of the C-2, C-3, and C-4 chiral centers. In contrast, we envisioned a novel enantioselective method (Scheme 1) that involves intramolecular aldol cyclization to generate key intermediate **5** using the self-regeneration of stereocenters (SRS) principle developed by Seebach et al.[12] to simultaneously generate these three chiral centers. Specif-

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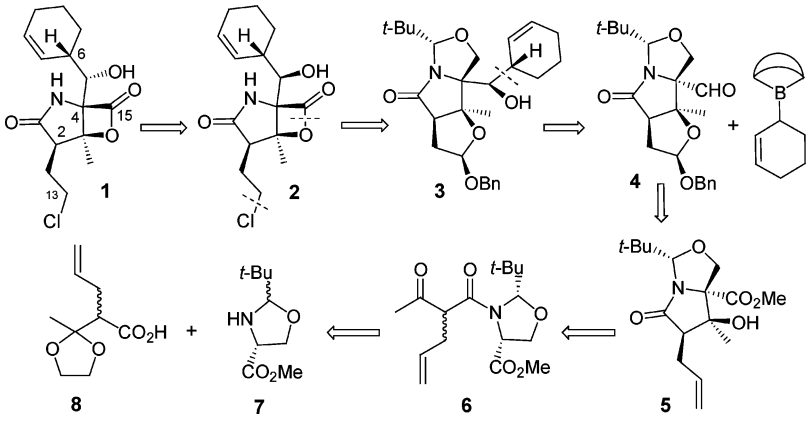
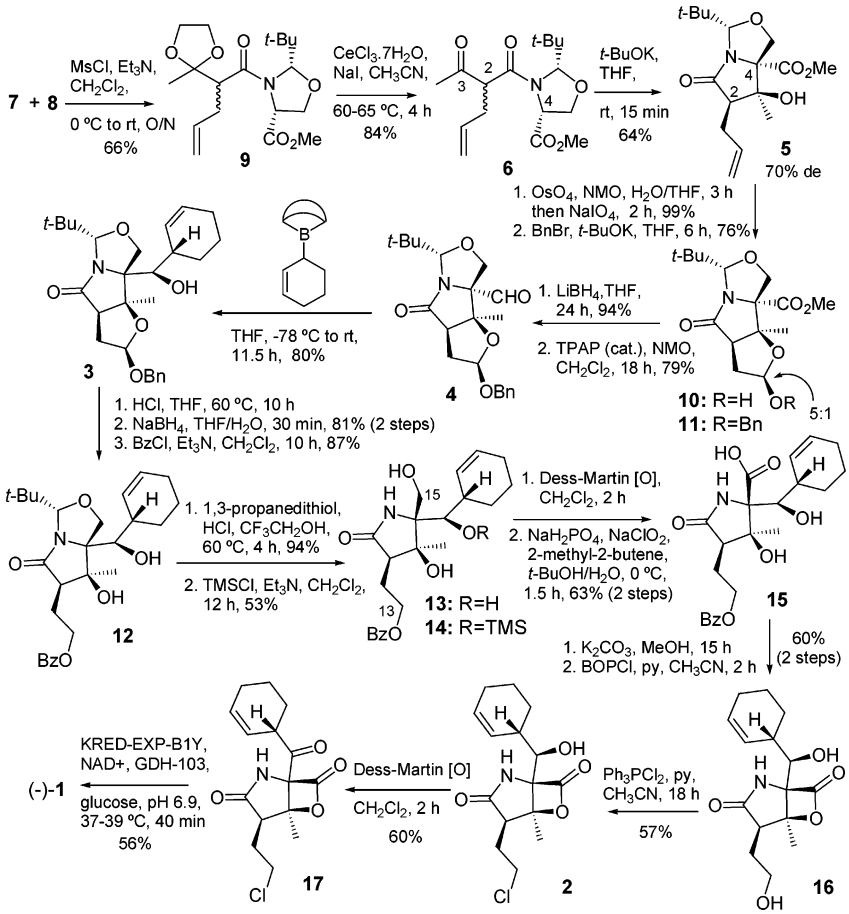
Scheme 1. Retrosynthetic Analysis of NPI-0052 (**1**)



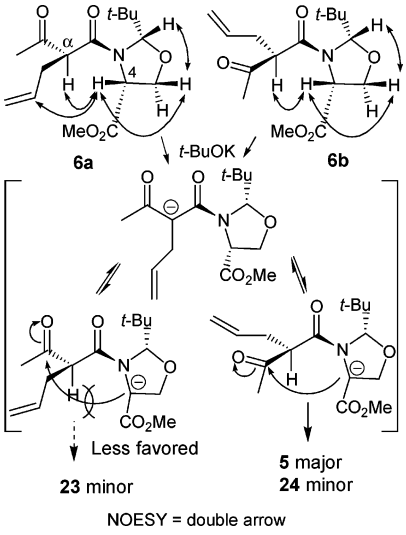
ically, we synthesized enantiomerically pure oxazolidine-γ-lactam **5** from β-keto amide **6**, where the C-4 chirality (derived from D-serine) is maintained during the intramolecular aldol cyclization following a strategy previously described by Andrews et al.,[13] and the C-2 and C-3 chiral centers are simultaneously constructed in a substrate-directed fashion (Scheme 1). The resulting, highly functionalized intermediate **5** served as a key precursor for the enantioselective total synthesis of **1**. Compound **5** was advanced to aldehyde **4**, to which the cyclohexene ring was installed using Brown's allylboration chemistry. The oxazolidine-protected alcohol was revealed and oxidized in preparation for β-lactone formation, followed by halogenation of the C-2 side chain to give the C-5 epimer of **1**. The final stereocenter was established at C-5 by oxidation followed by treatment with an enantioselective ketoreductase enzyme.[14]

Scheme 2. Synthesis of NPI-0052 (**1**)



The total synthesis (Scheme 2) commences with peptide coupling of **7** and **8** (see Supporting Information). Oxazolidine **7** (derived from D-serine[15]) serves as both a chiral directing group for the intramolecular aldol cyclization and a protecting group within the target oxazolidine-γ-lactam. While conventional peptide coupling conditions (e.g., DCC, EDAC) failed to provide the desired reaction between **7** and **8**, MsCl-mediated coupling conditions gave protected β-keto amide **9**. Deprotection of **9** generated β-keto amide **6** as a 3:2 mixture of epimers at the α-carbon bearing the allyl substituent (**6a** and **6b**, respectively). The relative stereochemistry was determined by analysis of a NOESY spectrum acquired on a mixture of **6a** and **6b** (Scheme 3), which

Scheme 3. Aldol Cyclization Mechanism



indicated that the *t*-Bu and methyl ester substituents adopt an exclusively *cis* relationship. This result is consistent with the established findings of Seebach et al., who demonstrated that 1:1 mixtures of *cis*- and *trans*-substituted oxazolidines yield pure *cis*-diastereomers upon *N*-acylation.[12] Similarly, Andrews et al. found that related L-serine-derived β-keto-amides were prepared largely as *cis* products **18** and **19**. Whereas intramolecular aldol cyclization of **19** gave **20** (~5% de), **21**, and **22** (Figure 2) in a ratio of 43:35:4,[13] in our case, **6** gives rise to **5** in 70% de (Figure 1), along with minor diasteromers **23**, **24**, and dehydration product **25** in a ratio of 71:2.5:10:16.5. **5** is routinely obtained as a pure enantiomer in at least 50% recovery from **6** after crystallization. We have successfully scaled all steps leading to this point in the synthesis to generate 100 g of **5**.

To explore the stereoselectivity of the intramolecular aldol reaction, the configurations of **6a** and **6b** were further considered (Scheme 3). The NOESY spectrum of the mixture of **6a** and **6b** indicated that in each case the α-protons are

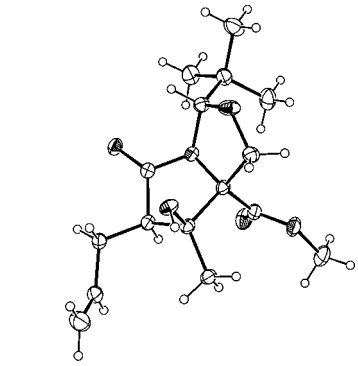


Figure 1. ORTEP plot of the X-ray crystal structure of **5**, depicting the absolute stereochemistry.

oriented toward C-4 and the oxygen of the amide carbonyl is pointing toward the *t*-Bu group; this is consistent with structural observations on related compounds.[12] In the case of **6a**, an additional NOE was observed from H-4 to the allyl proton. Upon formation of the enolate ion in the presence of base, the configuration of **6b** (as opposed to **6a**) favors intramolecular aldol cyclization, as the allyl group does not obstruct enolate addition to the β-keto carbonyl, thereby giving rise to **5** as the major product (with **24** as a minor product, also arising from **6b**). To further explore the stereoselectivity of this reaction, **6a** and **6b** were independently subjected to our cyclization conditions; interestingly, each gave **5** as the major product. This can be rationalized by enolate equilibration in the presence of base, allowing cyclization to the thermodynamically more stable product, as described by Andrews et al.[13] In our case, the allyl side chain adopts the less hindered relative configuration while C-4 retains its original absolute configuration (Scheme 3) based on SRS principle; thus, the *cis* relationship between the *t*-Bu and methyl ester substituents of the oxazolidine ring is maintained.

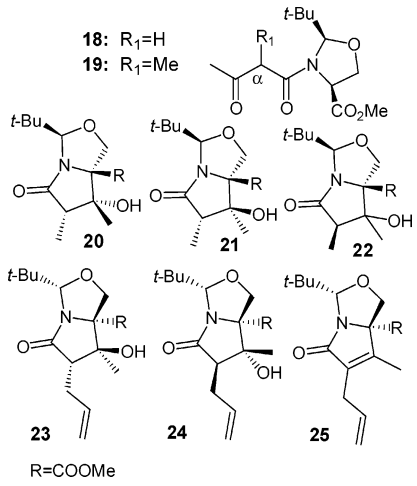


Figure 2. Structures for discussion.

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To progress **5** to (−)-**1**, the allyl group was oxidized with OsO_4/NMO in THF/H_2O followed by $NaIO_4$ to obtain hemiacetal **10** and its diastereomer, which were treated with BnBr in the presence of *t*-BuOK to produce **11** (and its diastereomer; 5:1). To set the stage for cyclohexene ring addition, the methyl ester of **11** was reduced to the corresponding primary alcohol, followed by TPAP/NMO oxidation to afford aldehyde **4**. Cyclohexene ring installation on **4** using Corey's method (with cyclohexenylzinc chloride)[7] indeed gave an *anti* addition product but with both undesired C-5 and C-6 stereocenters. This clearly distinguishes our oxazolidine-protected substrate **4** from the PMB-protected γ-lactam used in previous routes.[7,8,10] We therefore turned to Brown's allylboration chemistry (i.e., coupling of **4** with *B*-2-cyclohexen-1-yl-9-BBN[16]), which was expected to give a *syn* addition product. Fortunately, the product **3** had the desired stereochemistry at C-6, as established by X-ray; thus, the required C-5 stereocenter would need to be generated later. This was known to be feasible on the basis of our prior experience with semisynthetic transformations on the natural product.[3,14] In preparation for β-lactone formation, the C-13 benzyl acetal of **3** was replaced with a benzoyl protecting group (giving **12**) to allow it to withstand the aminal deprotection (strong acid conditions) and simultaneously differentiate the C-13 primary alcohol from the newly revealed C-15 primary alcohol (**13**), which must be selectively oxidized. Initial attempts to convert the C-15 primary alcohol of **13** directly to the corresponding carboxylic acid (in the presence of a free or acetylated C-5 hydroxyl group) under various oxidation conditions (TEMPO/BAIB, TEMPO/TCCA, Dess-Martin/$NaClO_2$, CrO_3/H_2SO_4) were unsuccessful, indicating that protection of the C-5 hydroxyl group is crucial. Gratifyingly, we realized that a TMS protecting group fits the need. TMS protection of C-5 hydroxyl followed by oxidation of the C-15 primary alcohol gave the corresponding carboxylic acid **15**. Deprotection of the benzoyl group of **15** followed by lactonization with BOPCl afforded the desired β-lactone **16**. Chlorination of lactone **16** with Ph_3PCl_2 afforded **2**, the C-5 epimer of **1**. The C-5 hydroxyl group of **2** was oxidized by Dess-Martin periodinane to provide ketone **17**, which was stereoselectively reduced by a ketoreductase enzyme[14] to afford (−)-**1** with no evidence of C-5 epimer **2**. The specific rotation and ^1H and ^{13}C NMR spectra of the synthetic sample of **1** are in good agreement with those of the natural product.

We have developed a novel enantioselective total synthesis of **1**. The key features include intramolecular aldol cyclization to simultaneously generate the three chiral centers of advanced intermediate **5**, cyclohexene ring addition using *B*-2-cyclohexen-1-yl-9-BBN, and inversion of the C-5 stereocenter by oxidation followed by enantioselective enzymatic reduction.[14] The synthesis of **5** is suitable for scale-up and involves no chromatography, except for the purification of **6**.

Supporting Information Available: Detailed experimental procedures and NMR spectra for all compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

OL0706051

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