

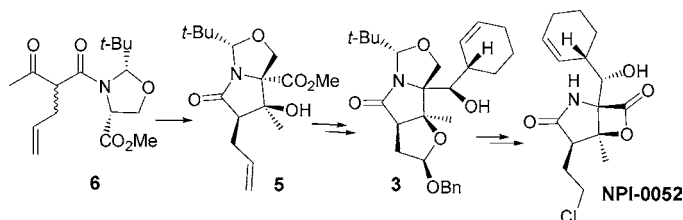
Enantioselective Total Synthesis of
(–)-Salinosporamide A (NPI-0052)Taotao Ling, Venkat R. Macherla,* Rama Rao Manam, Katherine A. McArthur, and
Barbara C. M. Potts

Nereus Pharmaceuticals, Inc., 10480 Wateridge Circle, San Diego, California 92121

vmacherla@nereuspharm.com

Received March 12, 2007

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 strategy⁷ 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-

(1) Feling, R. H.; Buchanan, G. O.; Mincer, T. J.; Kauffman, C. A.; Jensen, P. R.; Fenical, W. *Angew. Chem., Int. Ed.* **2003**, *42*, 355–357.

(2) Chauhan, D.; Catley, L.; Li, G.; Podar, K.; Hideshima, T.; Velankar, M.; Mitsiades, C.; Mitsiades, N.; Yasui, H.; Letai, A.; Ova, H.; Berkers, C.; Nicholson, B.; Chao, T.-H.; Neuteboom, S. T. C.; Richardson, P.; Palladino, M. A.; Anderson, K. C. *Cancer Cell* **2005**, *8*, 407–419.

(3) Macherla, V. R.; Mitchell, S. S.; Manam, R. R.; Reed, K. A.; Chao, T.-H.; Nicholson, B.; Deyanat-Yazdi, G.; Mai, B.; Jensen, P. R.; Fenical, W.; Neuteboom, S. T. C.; Lam, K. S.; Palladino, M. A.; Potts, B. C. M. *J. Med. Chem.* **2005**, *48*, 3684–3687.

(4) Groll, M.; Huber, R.; Potts, B. C. M. *J. Am. Chem. Soc.* **2006**, *128*, 5136–5141.

(5) Williams, P. G.; Buchanan, G. O.; Feling, R. H.; Kauffman, C. A.; Jensen, P. R.; Fenical, W. *J. Org. Chem.* **2005**, *70*, 6196–6203.

(6) Reed, K. A.; Manam, R. R.; Mitchell, S. S.; Xu, J.; Teisan, S.; Chao, T.-H.; Deyanat-Yazdi, G.; Neuteboom, S. T. C.; Lam, K. S.; Potts, B. C. M. *J. Nat. Prod.* **2007**, *70*, 269–276.

(7) (a) Reddy, L. R.; Saravanan, P.; Corey, E. J. *Am. Chem. Soc.* **2004**, *126*, 6230–6231. (b) Reddy, L. R.; Fournier, J.-F.; Reddy, B. V. S.; Corey, E. J. *Org. Lett.* **2005**, *7*, 2699–2701.

(8) Endo, A.; Danishefsky, S. J. *J. Am. Chem. Soc.* **2005**, *127*, 8298.

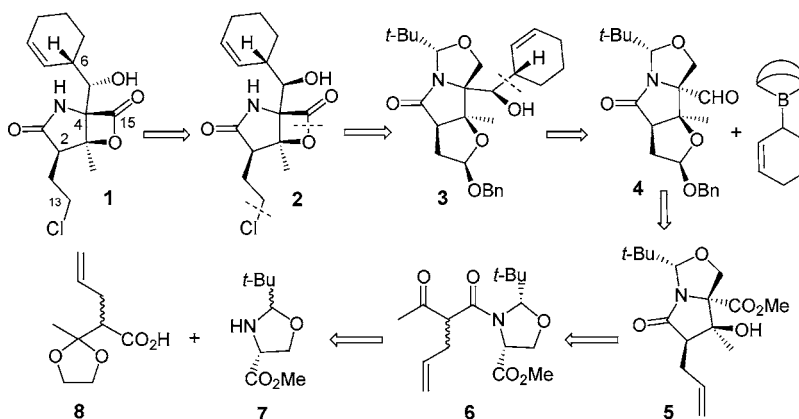
(9) (a) Caubert, V.; Masse, J.; Retailleau, P.; Langlois, N. *Tetrahedron Lett.* **2007**, *48*, 381–384. (b) Caubert, V.; Langlois, N. *Tetrahedron Lett.* **2006**, *47*, 4473–4475.

(10) Mulholland, N. P.; Pattenden, G.; Walters, I. A. S. *Org. Biomol. Chem.* **2006**, *4*, 2845–2846.

(11) Shibasaki, M.; Kanai, M.; Fukuda, N. *Chem. Asian J.* **2007**, *2*, 20–38.

(12) Seebach, D.; Sting, A. R.; Hoffmann, M. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 2708–2748.

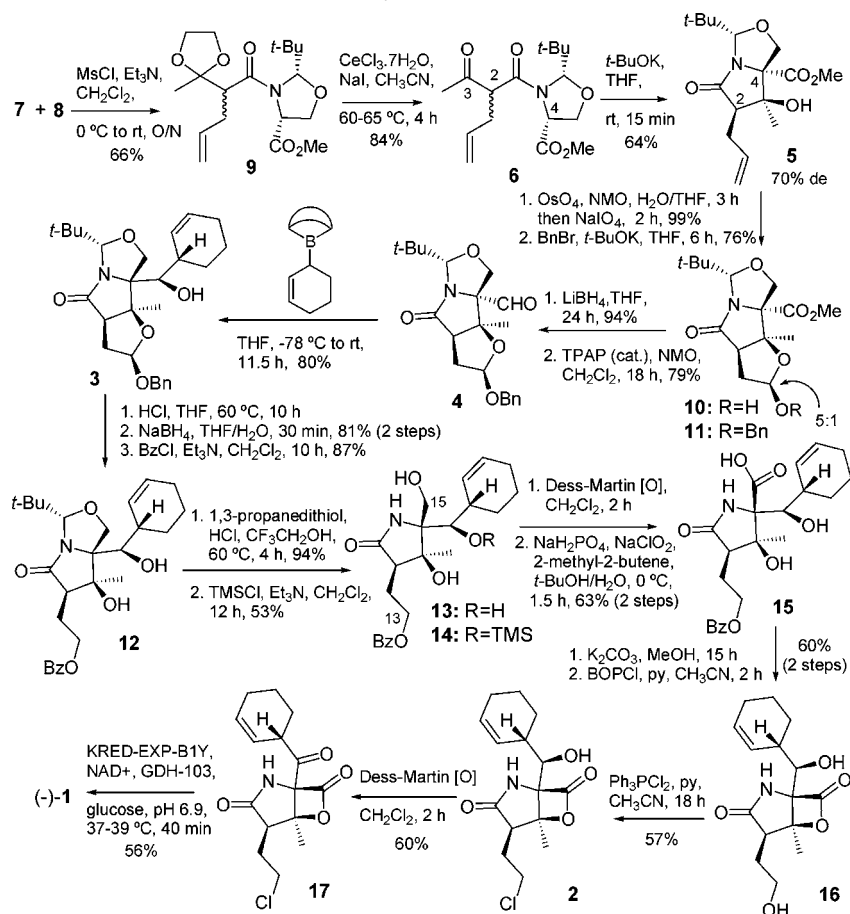
Scheme 1. Retrosynthetic Analysis of NPI-0052 (**1**)



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

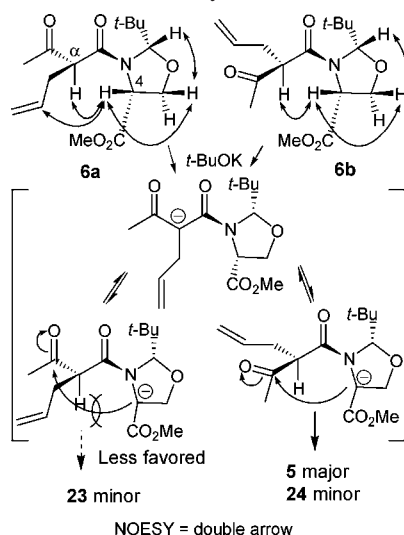
total synthesis of **1**. Compound **5** was advanced to aldehyde **4**, to which the cyclohexene ring was installed using Brown's allylboration chemistry. The oxazolidine-protected alcohol was revealed and oxidized in preparation for β -lactone formation, followed by halogenation of the C-2 side chain 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-serine¹⁵) 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 diastereomers **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

(13) Andrews, M. D.; Brewster, A. G.; Moloney, M. G. *Synlett*. **1996**, 612–614.

(14) Manam, R. R.; Macharla, V. R.; Potts, B. C. M. *Tetrahedron Lett.* **2007**, 48, 2537–2540. Improved yields have been reported herein.

(15) Seebach, D.; Abei, J. D. *Tetrahedron Lett.* **1984**, 25, 2545–2548.

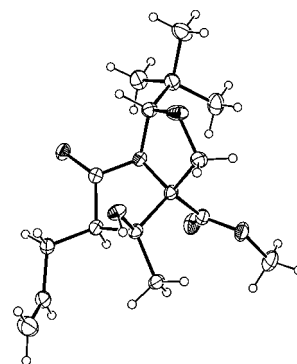


Figure 1. ORTEP plot of the X-ray crystal structure of **5**, depicting the absolute stereochemistry.

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.

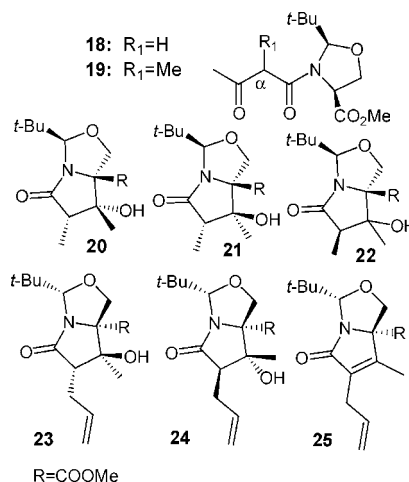


Figure 2. Structures for discussion.

To progress **5** to (–)-**1**, the allyl group was oxidized with OsO₄/NMO in THF/H₂O followed by NaIO₄ 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-BBN¹⁶), 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 amination 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/NaClO₂, CrO₃/H₂SO₄) 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 Ph₃PCl₂ 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 enzyme¹⁴ to afford (–)-**1** with no evidence of C-5 epimer **2**. The specific rotation and ¹H and ¹³C 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

(16) Kramer, G. W.; Brown, H. C. *J. Organomet. Chem.* **1977**, *132*, 9.