

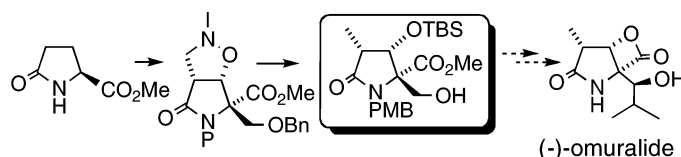
Nitrone [2+3]-Cycloadditions in Stereocontrolled Synthesis of a Potent Proteasome Inhibitor: (–)-Omuralide

Jean-Christophe Legeay and Nicole Langlois*

Institut de Chimie des Substances Naturelles, CNRS, Avenue de la Terrasse, 91198 Gif-sur-Yvette, France

nicole.langlois@icsn.cnrs-gif.fr

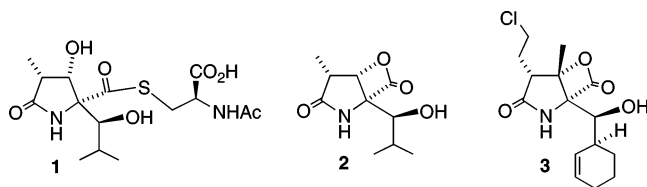
Received September 8, 2007



A new stereocontrolled synthetic route to omuralide has been developed from methyl pyroglutamate. This route involves regio- and stereoselective *N*-methylnitrone 1,3-dipolar cycloadditions to appropriate pyrrolinones, β -eliminations, and highly selective hydrogenations as the main steps.

Introduction

Lactacystin (**1**) was first isolated from a culture broth of *Streptomyces* sp. OM-6519.¹ It generates, by loss of *N*-acetylcysteine and lactonization, omuralide (**2**) [(–)-*clasto*-lactacystin], which is well-known to specifically inhibit the proteolytic activity of the proteasome 20S and represents the cell-permeable and biologically active form of **1**.² The proteasome, participating in a wide range of cellular processes, is responsible for the normal turnover of cellular proteins and recently became a novel target for cancer therapy.³ Structurally related salinosporamide A (**3**), isolated more recently from a



marine actinomycete, *Salinispora tropica*,⁴ is a still more effective proteasome inhibitor and a promising anticancer drug.⁵

(1) (a) Omura, S.; Fujimoto, T.; Otoguro, K.; Matsuzaki, K.; Moriguchi, R.; Tanaka, H.; Sasaki, Y. *J. Antibiot.* **1991**, *44*, 113–116. (b) Omura, S.; Matsuzaki, K.; Fujimoto, T.; Kosuge, K.; Furuya, T.; Fujita, S.; Nakagawa, A. *J. Antibiot.* **1991**, *44*, 117–118.

(2) (a) Fenteany, G.; Standaert, R. F.; Reichard, G. A.; Corey, E. J.; Schreiber, S. L. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 3358–3362. (b) Dick, L. R.; Cruikshank, A. A.; Grenier, L.; Melandri, F. D.; Nunes, S. L.; Stein, R. L. *J. Biol. Chem.* **1996**, *271*, 7273–7275.

(3) (a) Borissenko, L.; Groll, M. *Chem. Rev.* **2007**, *107*, 687–717. (b) Voorhees, P. M.; Dees, E. C.; O'Neil, B.; Orłowski, R. Z. *Clin. Cancer Res.* **2003**, *9*, 6316–6325. (c) Spano, J.-P.; Bay, J.-O.; Blay, J.-Y.; Rixe, O. *Bull. Cancer* **2005**, *92*, 945–952.

(4) 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.

Indeed, while bortezomib is the first proteasome inhibitor approved by the FDA for the treatment of multiple myeloma,⁶ salinosporamide A (NPI-0052) is currently in clinical trials, due to its oral activity against bortezomib-resistant multiple myeloma cells,⁷ and the discovery of its biological activities renders omuralide analogues attractive synthetic targets.

These small but complex molecules are composed of a highly functionalized pyrrolidinone- β -lactone bicyclic core. The presence of three chiral centers on the γ -lactam ring, with a quaternary asymmetric carbon at the β -lactone–ring junction in the α -position to the nitrogen, constitutes structural features common to **1–3** and related compounds. Since the pioneering work of E. J. Corey,⁸ numerous lactacystin or omuralide syntheses have been described to date, and several reviews have been devoted to them.^{9,10} To synthesize salinosporamide A, different strategies have already been developed by five groups.¹¹ Most of them involve an introduction of the cyclohexenyl side chain from an aldehyde intermediate at a late stage,

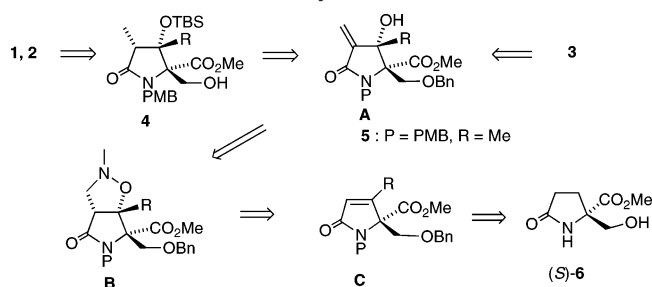
(5) (a) Macherla, V. R.; Mitchell, S. S.; Manam, R. R.; Reed, K. A.; Chao, T.-H.; Nicholson, B.; Deyanat-Yazdi, G.; Mai, B.; Jensen, P. R.; Fenical, W. F.; Neuteboom, S. T. C.; Lam, K. S.; Palladino, M. A.; Potts, B. C. M. *J. Med. Chem.* **2005**, *48*, 3684–3687. (b) Groll, M.; Huber, R.; Potts, B. C. M. *J. Am. Chem. Soc.* **2006**, *128*, 5136–5141.

(6) (a) Adams, J. *Drug Discovery Today* **2003**, *8*, 307–315. (b) Rajkumar, S. V.; Richardson, P. G.; Hideshima, T.; Anderson, K. C. *J. Clin. Oncol.* **2005**, *23*, 630–639.

(7) (a) Chauhan, D.; Catley, L.; Li, G.; Podar, K.; Hideshima, T.; Velankar, M.; Mitsiades, C.; Mitsiades, N.; Yasui, H.; Letai, A.; O'vaa, H.; Berkers, C.; Nicholson, N.; Chao, T.-H.; Neuteboom, S. T. C.; Richardson, P.; Palladino, M. A.; Anderson, K. C. *Cancer Cell* **2005**, *8*, 407–419. (b) Joazeiro, C. A. P.; Anderson, K. C.; Hunter, T. *Cancer Res.* **2006**, *66*, 7840–7842.

(8) (a) Corey, E. J.; Reichard, G. A. *J. Am. Chem. Soc.* **1992**, *114*, 10677–10678. (b) Corey, E. J.; Reichard, G. A.; Kania, R. *Tetrahedron Lett.* **1993**, *34*, 6977–6980. (c) Corey, E. J.; Li, W.; Nagamitsu, T. *Angew. Chem., Int. Ed.* **1998**, *37*, 1676–1679.

SCHEME 1. General Retrosynthetic Route

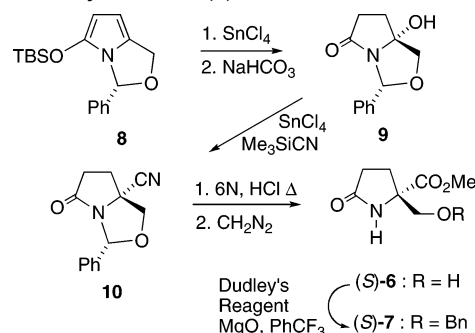
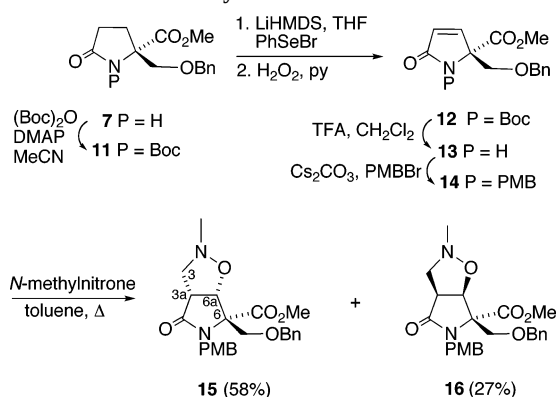


relying on Corey et al. strategies.^{11a,b} These pathways, indeed, allow structural modifications of the side chain α to the nitrogen, and this advantage has been exemplified by this group in several syntheses of salinosporamide A as well as omuralide analogues.^{8c,12}

Results and Discussion

We planned to develop a general approach to **1–3** and to several natural or synthetic analogues from particularly inexpensive methyl pyroglutamate, with the aim to synthesize the key advanced intermediates **4** and **5** reported by Corey, following the retrosynthetic Scheme 1. Accordingly, the densely substituted pyrrolidinone **4** would be generated by stereoselective hydrogenation of 4-*exo*-methylene precursor **A** ($R = H$). 4-*exo*-methylene-5-oxopyrrolidines of general structure **A** would be obtained in a few steps through β -elimination of an amino group formed from **B**, via the reductive opening of the isoxazolidine ring. Indeed, we previously reported preliminary studies of this new strategy leading to salinosporamide A precursor **5**,¹³ and we expected that versatile *exo*-methylene derivative **A** ($R = H$) could also be converted into 3-hydroxy-4-methylpyrrolidin-2-ones **4** with the *cis* configuration present in omuralide (**2**). We anticipated that requisite functionalities

SCHEME 2. Synthesis of (S)-7


 SCHEME 3. Preparation of Dipolarophile 14 and Cycloaddition of *N*-Methylnitron


of **B** could be introduced with appropriate configurations through regio- and stereoselective *N*-alkylnitron cycloadditions to unsaturated γ -lactams **C**, already bearing the quaternary stereogenic center C-2. The preparation of **C** could take advantage of the original access to (*S*)-methyl 2-(hydroxymethyl)pyroglutamate [(*S*)-**6**], previously developed in our laboratory to synthesize deoxydisibetaine.¹⁴ Thus, we selected as a synthetic target compound **4**, which has been converted into **2** by Corey et al.,^{8c} Pattenden et al.,^{10a} and very recently Kobayashi et al.¹⁰ⁱ We reported therein the pathways and results to achieve this goal. The scope, efficiency, and stereoselectivity of this new route were investigated starting with methyl 2-[(benzyloxy)methyl]pyroglutamate (**7**), and we showed that the results depend to a large extent on the protection of functional groups and on the order in which the main steps are performed. Racemic **7** was directly obtained from methyl pyroglutamate¹³ and used to check the validity and efficiency of our routes.

(*S*)-Methyl 2-[(benzyloxy)methyl]pyroglutamate [(*S*)-**7**] was prepared from bicyclic [(*tert*-butyldimethylsilyl)oxy]pyrrole **8** derived from (*S*)-pyroglutaminol as summarized in Scheme 2.¹⁴ This pathway involved the formation of the nitrile **10** with control of the stereogenic quaternary center by a selective addition of trimethylsilyl cyanide to the acyliminium ion generated from tertiary carbinolamide **9**.¹⁵ Acidic hydrolysis of **10**, followed by methylation with diazomethane, gave rise to (*S*)-**6**, which was selectively O-benzylated to (*S*)-**7** (75%) with 2-(benzyloxy)-1-methylpyridinium triflate (Dudley's reagent) in the presence of MgO.¹⁶ It is worthy of note that neither *N*-benzylation nor transesterification with the methoxycarbonyl

(9) For reviews see: (a) Corey, E. J.; Li, W.-D. *Z. Chem. Pharm. Bull.* **1999**, *47*, 1–10. (b) Masse, C. E.; Morgan, A. J.; Adams, J.; Panek, J. S. *Eur. J. Org. Chem.* **2000**, 2513–2528. (c) Kang, S. H.; Kang, S. Y.; Lee, H.-S.; Buglass, A. J. *Chem. Rev.* **2005**, *105*, 4537–4558. (d) Shibasaki, M.; Kanai, M.; Fukuda, N. *Chem. Asian J.* **2007**, *2*, 20–38.

(10) For recent syntheses see: (a) Brennan, C. J.; Pattenden, G.; Rescouri, G. *Tetrahedron Lett.* **2003**, *44*, 8757–8760. (b) Ooi, H.; Ishibashi, N.; Iwabuchi, Y.; Ishihara, J.; Hatakeyama, S. *J. Org. Chem.* **2004**, *69*, 7765–7768. (c) Donohoe, T. J.; Sintim, H. O.; Sisangia, L.; Ace, K. W.; Guyo, P. M.; Cowley, A.; Harling, J. D. *Chem.-Eur. J.* **2005**, *11*, 4227–4238. (d) Wardrop, D. J.; Bowen, E. G. *Chem. Commun.* **2005**, 5106–5108. (e) Hayes, C. J.; Sherlock, A. E.; Selby, M. D. *Org. Biomol. Chem.* **2006**, *4*, 193–195. (f) Fukuda, N.; Sasaki, K.; Sastry, T. V. R. S.; Kanai, M.; Shibasaki, M. *J. Org. Chem.* **2006**, *71*, 1220–1225. (g) Balskus, E. P.; Jacobsen, E. N. *J. Am. Chem. Soc.* **2006**, *128*, 6810–6812. (h) Yoon, C. H.; Flanagan, D. L.; Yoo, K. S.; Jung, K. W. *Eur. J. Org. Chem.* **2007**, 37–39. (i) Gilley, C. B.; Buller, M. J.; Kobayashi, Y. *Org. Lett.* **2007**, *9*, 3631–3636.

(11) (a) Reddy, L. R.; Saravanan, P.; Corey, E. J. *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. (c) Endo, A.; Danishefsky, S. J. *J. Am. Chem. Soc.* **2005**, *127*, 8298–8299. (d) Mulholland, N. P.; Pattenden, G.; Walters, I. A. S. *Org. Biomol. Chem.* **2006**, *4*, 2845–2846. (e) Ma, G.; Nguyen, H.; Romo, D. *Org. Lett.* **2007**, *9*, 2143–2146. (f) Ling, T.; Macherla, V. R.; Manam, R. R.; McArthur, K. A.; Potts, B. C. M. *Org. Lett.* **2007**, *9*, 2289–2292.

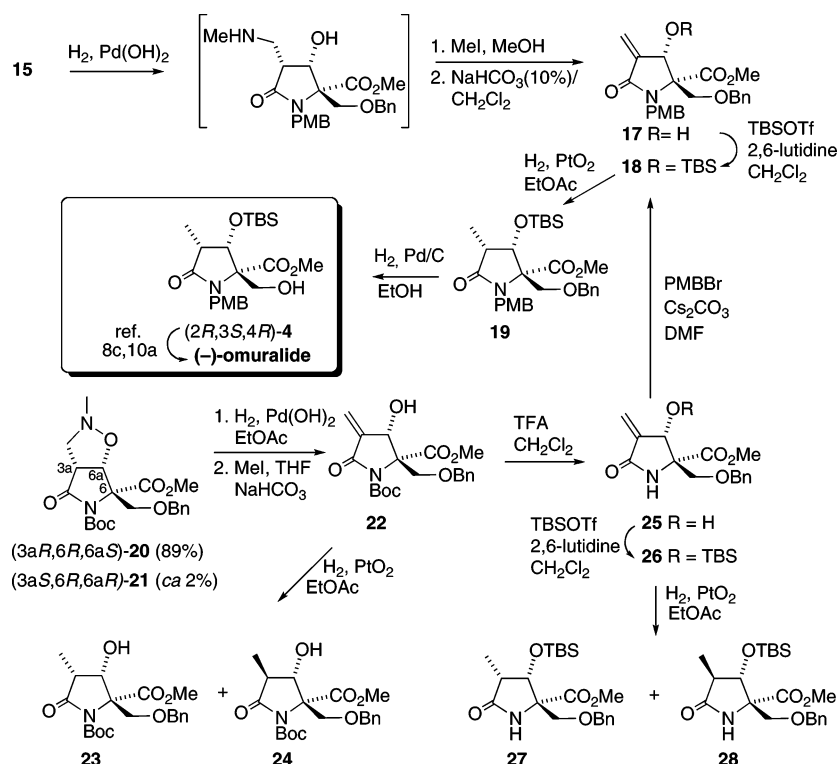
(12) Corey, E. J.; Li, W.-D. Z.; Nagamitsu, T.; Fenteany, G. *Tetrahedron* **1999**, *55*, 3305–3316.

(13) (a) Caubert, V.; Langlois, N. *Tetrahedron Lett.* **2006**, *47*, 4473–4475. (b) Caubert, V.; Massé, J.; Retailleau, P.; Langlois, N. *Tetrahedron Lett.* **2007**, *48*, 381–384. (c) Legeay, J.-C.; Langlois, N. XI Symposium ICSN, Gif-sur-Yvette, France, June 7, 2007.

(14) Langlois, N.; Le Nguyen, B. K. *J. Org. Chem.* **2004**, *69*, 7558–7564.

(15) Langlois, N.; Choudhury, P. K. *Tetrahedron Lett.* **1999**, *40*, 2525–2528.

SCHEME 4. Synthesis of 4



group was observed under these particular conditions. Compound **7** was transiently *N*-protected as a *tert*-butyl carbamate (**11**) (100%) to enhance the reactivity of lactam carbonyl, allowing efficient deprotonation at C-4 and introduction of the conjugate double bond of **12** (89%, Scheme 3).

In a first route, the *N*-Boc group of ($\pm$)-**12** was quantitatively removed, providing **13**, and the PMB group present in the target molecule was introduced ((PMB)Br, Cs₂CO₃, DMF, rt) to give the dipolarophile **14** in 88% yield. The 1,3-dipolar cycloaddition of *N*-methylnitrone to **14** proceeded by heating in toluene with high regioselectivity but rather moderate stereoselectivity. This addition was complete after 3 h, affording two main adducts, **15** and **16**, isolated in 85% combined yield and a ratio near 2.1:1. In the NMR spectra of **15** and **16**, proton and carbon chemical shifts at the bridgehead positions 3a (CDCl₃; ¹H, 3.52 and 3.46 ppm; ¹³C, 51.9 and 51.0 ppm, respectively) support the same regiochemistry in the cycloaddition.¹⁷ An NOESY experiment performed with the major diastereomer **15** showed a weak correlation between H-6a and one proton of the oxymethylene group at C-6. The configurations consequently attributed were confirmed later by further chemical correlations.

The isoxazolidine **15** was hydrogenolyzed using Pearlman's catalyst,¹⁸ with complete chemoselectivity, leaving the benzyloxy protecting group untouched. The cleavage product was converted into trimethylammonium iodide as the leaving group by MeI in MeOH and directly submitted to a β -elimination in a basic biphasic mixture (CH₂Cl₂–aqueous 10% (w/v) Na₂CO₃) to afford compound **17** in 67% yield for this three-step procedure (Scheme 4). Conversion of **17** into its TBS ether **18** with (TBS)-

Otf and 2,6-lutidine (not optimized, 73% yield), followed by highly stereoselective hydrogenation furnished **19**, the direct precursor of **4**, as the sole observed diastereomer (dr > 95:5). The expected compound **4**, an intermediate in several syntheses of omuralide (**2**),^{8c,10a,i} was isolated in 89% yield after quantitative O-debenzylation. Direct conversion of **18** into **4** was also obtained (87%) under the same conditions (10% Pd/C, EtOH).

Despite the excellent stereoselectivity of the hydrogenation step, the efficiency of the general Scheme 1 could be improved by increasing the stereoselectivity of the *N*-methylnitrone cycloaddition to the flat pyrrolinone ring. The modest facial selectivity observed with **14** could be explained by a relatively weak steric hindrance exerted by the side chain at C-2. Indeed, the 2-(benzyloxy)methyl group could adopt a conformation minimizing the congestion and allowing an attack of the nitrone from the same face, plausibly due to implication of the *N*-PMB group in intramolecular π -stacking.

Accordingly, we investigated a second route starting with *N*-Boc-lactam (*S*)-**12** as the dipolarophile. The 1,3-dipolar cycloaddition is effectively much more selective in this case, leading to the adduct (3a*R*,6*R*,6a*S*)-**20** in 89% yield. The stereostructure **20** was assigned on the basis of NMR data, including an NOESY experiment. Only ca. 2% of another diastereoisomer was isolated, whose spectral data support structure **21**. After hydrogenolysis of the isoxazolidine moiety, the corresponding trimethylammonium iodide obtained with excess MeI in THF was treated in one pot with anhydrous NaHCO₃, affording the 4-*exo*-methylene derivative **22** in 89% yield for the three steps (from **20**, Scheme 4).

Hydrogenation of the *exo*-methylene double bond of **22**, with PtO₂ as the catalyst, occurred quantitatively but without any selectivity, leading to **23** and **24** in a ratio near 1:1 determined by ¹H NMR (Table 1, entry 1). The lack of selectivity could be due to the much decreased bulk of the vicinal C-3 substituent,

(16) Poon, K. W. C.; Dudley, G. B. *J. Org. Chem.* **2006**, *71*, 3923–3927.

(17) Langlois, N.; Van Bac, N.; Delcroix, J.-M.; Deyne, A.; Griffart-Brunet, D.; Chiaroni, A.; Riche, C. *Tetrahedron* **1995**, *51*, 3571–3586.

(18) Pearlman, W. M. *Tetrahedron Lett.* **1967**, 1663–1664.

TABLE 1. Hydrogenation of 4-*exo*-Methylene Derivatives

Entry		H ₂ / EtOAc Catalyst		
			(ratio) ^a	
1	22 R ₁ = Boc, R ₂ = H	PtO ₂	23 (48)	24 (52)
2	22 R ₁ = Boc, R ₂ = H	Pd(OH) ₂	23 (62)	24 (38)
3	26 R ₁ = H, R ₂ = TBS	PtO ₂	27 (90)	28 (10)
4	18 R ₁ = PMB, R ₂ = TBS	PtO ₂	19 (>95)	(<5)

^a Determined by ¹H NMR of the crude diastereomeric mixture obtained in 99–100% yield, except for entry 2 (40%).

but also to its directing effect. Indeed, hydroxyl groups are known to enforce addition of hydrogen from their own side of the molecule.¹⁹ This result indicated that the cleavage of the isoxazolidine moiety of cycloadduct **20** has to be carefully controlled since some β -elimination of methylamine and subsequent nonstereoselective hydrogenation could occur in situ after a prolonged time under the hydrogenolysis conditions (Table 1, entry 2). It led us also to change the chronology of the O-protection step by *tert*-butyldimethylsilylation. Although *N*-Boc protective groups are known to be generally sensitive to (TBS)OTf under O-silylation conditions,²⁰ sequential *N*-deprotection followed by O-silylation was preferred. In this case, *N*-deprotection of **22** under mild conditions using ZnBr₂²¹ was less efficient than classical treatment with TFA in CH₂Cl₂, affording quantitatively compound **25**, which was O-silylated into **26** (97%, Scheme 4). Hydrogenation of **26** (PtO₂, EtOAc) proceeded with good selectivity (9:1; see Table 1, entry 3), providing the requisite diastereomer **27**, which was isolated in 77% yield. However, this compound was shown to be poorly reactive toward *N*-*p*-methoxybenzylation into **19** ((PMB)Br, Cs₂CO₃, DMF, rt), and only 40% conversion was obtained after 24 h. Consequently, this step was performed at an early stage, with the flattened and less hindered 4-*exo*-methylenepyrrolidinone **26**, leading to (2*R*,3*S*)-**18** (92%), the precursor of (–)-omuralide via (2*R*,3*S*,4*R*)-**19** and (2*R*,3*S*,4*R*)-**4**, as shown previously. Enantiomeric purity of (2*R*,3*S*,4*R*)-**4** (96% ee) was verified by chiral HPLC and indicated no significative lowering during the synthesis. Thus, by this second route, the overall yield of the key intermediate **4** from *N*-Boc-2-[(benzyloxy)methyl]-2-(methoxycarbonyl)pyrrolidin-2-one [(*S*)-**12**] was dramatically improved and reached 64%, compared to 22% obtained in the first route.

Conclusions

We have developed a new synthetic route to (–)-omuralide (**2**) from cheap methyl pyroglutamate. *N*-Methylnitron 1,3-dipolar cycloaddition to the *N*-Boc-pyrrolidinone already bearing the α,α -disubstituted stereogenic center, as described in the second route, was shown to be remarkably efficient, allowing achievement of the synthesis of the key omuralide precursor **4** with excellent stereoselectivities and high yields, provided that the *N*-Boc dipolarophile was used and that *N*-*p*-methoxybenzylation was performed before hydrogenation of the 4-*exo*-

methylene moiety. This strategy could also be broadened to the synthesis of structural analogues using substituted nitrones. Along with elaboration of the side chain α to the nitrogen at a late stage of the synthesis according to the versatile Corey procedures,^{8c,11a,b,12} it could also offer additional possibilities for variation of substituent α to the lactam carbonyl. It opens the way to other members of the salinosporamide–cinnabaramide family,^{22,23} as well as to the design of synthetic related compounds interesting to improve the selectivity of proteasome inhibition.^{10g,24}

Experimental Section

(*S*)-Methyl 2-[(Benzyloxy)methyl]-5-oxopyrrolidine-2-carboxylate ((*S*)-**7**) from (*S*)-Methyl 2-(Hydroxymethyl)-5-oxopyrrolidine-2-carboxylate [(*S*)-**6**]. A mixture of (*S*)-**6** (320 mg, 1.85 mmol),¹⁴ 2-(benzyloxy)-1-methylpyridinium triflate (1.40 g, 4.01 mmol), and dried MgO (163.4, 4.05 mmol) in (trifluoromethyl)-benzene (3.8 mL) was stirred at 83 °C for 21 h before filtration through Celite. The Celite was washed with CH₂Cl₂, and the solvents were evaporated. The product was purified by column chromatography (eluent heptane–EtOAc, 3:7) to give O-benzylated compound (*S*)-**7** (363 mg, 75%). [α]_D²⁵ +25.7 (c 1.72, CHCl₃). For spectral data see ref 13b. Anal. Calcd for C₁₄H₁₇NO₄: C, 63.86; H, 6.51; N, 5.32. Found: C, 63.56; H, 6.52; N, 5.37.

(*S*)-1-*tert*-Butyl 2-Methyl 2-[(Benzyloxy)methyl]-5-oxo-pyrrolidine-1,2-dicarboxylate [(*S*)-**11**]. A mixture of (*S*)-**7** (342 mg, 1.3 mmol), DMAP (158.6 mg, 1.3 mmol), and Boc₂O (437 mg, 2.0 mmol) in MeCN (1.6 mL) was stirred at rt under argon for 1 h and then concentrated under vacuum (without heating). The crude orange oil was purified by column chromatography (eluent petroleum ether–EtOAc, 65:35) to yield (*S*)-**11** as a colorless oil (472 mg, 100%). [α]_D²⁵ –40.2 (c 1.93, CHCl₃). IR (cm^{–1}): 2978, 1788, 1742, 1713, 1454, 1368, 1300, 1256. MS (ESI, MeOH, *m/z*): 386 [(MNa)⁺, 100], 286. ¹H NMR (500 MHz, CDCl₃): δ 7.33 (m, 2H), 7.28 (m, 3H), 4.55 (d, 1H, *J* = 12.2 Hz), 4.53 (d, 1H, *J* = 12.2 Hz), 4.07 (d, 1H, *J* = 10 Hz), 3.86 (d, 1H, *J* = 10 Hz), 3.73 (s, 3H), 2.75 (m, 1H), 2.50 (m, 1H), 2.29 (m, 1H), 2.08 (m, 1H), 1.43 (s, 9H). ¹³C NMR (75 MHz, CDCl₃): δ 174.4, 171.8, 149.2, 137.7, 128.6, 127.9, 127.7, 83.7, 73.6, 71.2, 68.1, 52.5, 31.4, 28.0, 27.2. Anal. Calcd for C₁₉H₂₅NO₆: C, 62.80; H, 6.93; N, 3.85. Found: C, 62.41; H, 6.84; N, 3.74.

(*S*)-1-*tert*-Butyl 2-Methyl 2-[(Benzyloxy)methyl]-5-oxo-1*H*-pyrrole-1,2-(2*H*,5*H*)-dicarboxylate [(*S*)-**12**]. LiHMDS (1 M in THF, 1.4 mL) was added dropwise to a solution of (*S*)-**11** (421 mg, 1.16 mmol) in THF (13.3 mL) and the mixture stirred under argon at –78 °C. After 30 min of stirring, PhSeCl (244.2 mg, 1.28 mmol) in THF (1.16 mL) was added. The mixture was stirred for 1.5 h at –78 °C, and satd NH₄Cl was added. Back at rt, the reaction mixture was extracted four times with EtOAc. The organic phase was dried on MgSO₄ and evaporated under vacuum. The residue was taken up in CH₂Cl₂ (7.5 mL) and pyridine (283 μ L), and H₂O₂ (30% aq, 1.24 mL) was added after the mixture was cooled at 0 °C. The mixture was stirred at 0 °C for 30 min and then at rt for 1.75 h. After addition of aqueous Na₂CO₃ (10%, w/v) and CH₂Cl₂ under stirring, the organic phase was separated and washed by water. The aqueous phase was extracted three times with CH₂Cl₂, and the organic layers were washed twice with H₂O, gathered, dried

(19) Thompson, H. W.; Rashid, S. Y. *J. Org. Chem.* **2002**, *67*, 2813–2825 and references therein.

(20) Kocienski, P. J. *Protecting Groups*, 3rd ed.; Thieme: Stuttgart, New York, 2005.

(21) (a) Nigam, S. C.; Mann, A.; Taddei, M.; Wermuth, C.-G. *Synth. Commun.* **1989**, *19*, 3139–3142. (b) Donohoe, T. J.; Chiu, J. Y. K.; Thomas, R. E. *Org. Lett.* **2007**, *9*, 421–424.

(22) Stadler, M.; Bitzer, J.; Mayer-Bartschmid, A.; Müller, H.; Benet-Buchholz, J.; Gantner, F.; Tichy, H.-V.; Reinemer, P.; Bacon, K. B. *J. Nat. Prod.* **2007**, *70*, 246–252.

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

(24) Soucy, F.; Grenier, L.; Behnke, M. L.; Destree, A. T.; McCormack, T. A.; Adams, J.; Plamondon, L. *J. Am. Chem. Soc.* **1999**, *121*, 9967–9976.

over MgSO_4 , and concentrated under vacuum. The residue was filtered on silica gel (eluent heptane– Et_2O , 1:9) to give (*S*)-**12** as a colorless oil (373.5 mg, 89%). $[\alpha]_{\text{D}}^{27} +56$ (c 1.36, CHCl_3). IR (cm^{-1}): 1782, 1739, 1711, 1319, 1254, 1158, 1102, 1049, 821. MS (ESI, MeCN, *m/z*): 745 (2MNa^+), 384 (MNa^+ , 100). ^1H (500 MHz, CDCl_3): δ 7.37–7.24 (m, 5H), 6.99 (d, 1H, $J = 6.1$ Hz), 6.24 (d, 1H, $J = 6.1$ Hz), 4.53 (d, 1H, $J = 12.2$ Hz), 4.51 (d, 1H, $J = 12.2$ Hz), 4.17 (d, 1H, $J = 10.4$ Hz), 4.14 (d, 1H, $J = 10.4$ Hz), 3.73 (s, 3H), 1.47 (s, 9H). ^{13}C NMR (75 MHz, CDCl_3): δ 169.0, 167.8, 148.6, 147.0, 137.5, 128.6, 128.4, 128.0, 127.7, 83.8, 73.8, 73.2, 68.6, 53.0, 28.0. Anal. Calcd for $\text{C}_{19}\text{H}_{23}\text{NO}_6$: C, 63.15; H, 6.41, N, 3.88. Found: C, 63.21; H, 6.41, N, 3.71.

Cycloaddition of *N*-Methylnitrone to ($\pm$)-14**: Methyl 6-[(Benzyloxy)methyl]-5-(4-methoxybenzyl)-2-methyl-4-oxohexahydro-2H-pyrrolo[3,4-*d*]isoxazole-6-carboxylates ($\pm$)-**15** and ($\pm$)-**16**.** A solution of *N*-methylnitrone (44.3 mg, 0.75 mmol) in dry toluene (2.5 mL) was added to ($\pm$)-**14** (111.7 mg, 0.29 mmol) placed under Ar. The mixture was stirred at 110 °C for 3 h. The solvent was evaporated under reduced pressure and the crude material purified by chromatography (eluent EtOAc) to give a mixture of isomers (127.7 mg, 99%). The diastereomeric ratio (58:28:8:5) was determined by HPLC (SunFire, 3 $\times$ 150 mm; eluent MeCN– H_2O , 1:1). The two main diastereomers were separated by preparative TLC (eluent CH_2Cl_2 –EtOAc, 9:1) to afford (*3aR**,*6R**,*6aS**)-**15** (75.0 mg, 58%) and (*3aS**,*6R**,*6aR**)-**16** (35 mg, 27%), described in the Supporting Information). ($\pm$)-**15** was obtained as a colorless gum. IR (cm^{-1}): 2953, 2848, 1753, 1692, 1512. MS (ESI, MeOH + CH_2Cl_2 , *m/z*): 463 (MNa^+ , 100). ^1H NMR (500 MHz, CDCl_3): δ 7.30–7.24 (m, 5H), 7.08 (d, 2H, $J = 7.4$ Hz), 6.75 (2H, d, 2H, $J = 8.5$ Hz), 5.19 (br d, 1H, $J = 15.7$ Hz), 4.66 (br d, 1H, $J = 6.3$ Hz), 4.12 (d, 1H, $J = 15.7$ Hz), 3.94 (d, 1H, $J = 11.4$ Hz), 3.86 (d, 1H, $J = 11.4$ Hz), 3.81 (masked d, 1H), 3.79 (s, 3H), 3.74 (s, 3H), 3.63 (m, 1H), 3.52 (m, 1H), 3.25 (d, 1H, $J = 9.8$ Hz), 2.65 (s, 3H), 2.56 (m, 1H). ^{13}C NMR (125 MHz, CDCl_3): δ 175.0, 168.2, 159.0, 137.6, 130.1, 129.9, 128.5, 127.8, 127.3, 113.7, 79.8, 76.0, 72.9, 70.3, 60.1, 55.4, 52.5, 51.9, 45.3, 44.9. HRMS (ESI, MeOH + CH_2Cl_2 , *m/z*): calcd for $\text{C}_{24}\text{H}_{28}\text{N}_2\text{O}_6\text{Na}$ (MNa^+) 463.1845, found 463.1882.

Cycloaddition of *N*-Methylnitrone to (*S*)-12**: 5-*tert*-Butyl 6-Methyl 6-[(Benzyloxy)methyl]-2-methyl-4-oxohexahydro-5H-pyrrolo[3,4-*d*]isoxazole-5,6-dicarboxylates (*3aR*,*6R*,*6aS*)-**20** and (*3aS*,*6R*,*6aR*)-**21**.** A solution of *N*-methylnitrone (58.0 mg, 0.98 mmol) in dry toluene (2.0 mL) was added to (*S*)-**12** (167.4 mg, 0.46 mmol) placed under Ar. The mixture was stirred at 110 °C for 2.2 h. The solvent was evaporated under reduced pressure, and the crude material was purified by chromatography (eluent heptane–EtOAc, 4:6) to give the main cycloadduct (*3aR*,*6R*,*6aS*)-**20** (173.3 mg, 89%) as a colorless gum and diastereomer (*3aS*,*6R*,*6aR*)-**21** (4.3 mg, 2.2% described in the Supporting Information). The following are data for (*3aR*,*6R*,*6aS*)-**20**. $[\alpha]_{\text{D}}^{24} +5.6$ (c 1.55, CHCl_3). IR (cm^{-1}): 2922, 1787, 1718, 1454, 1368, 1298, 1233, 1152, 1124, 1076. MS (ESI, MeOH, *m/z*): 443 (MNa^+), 343 [(*M* – Boc) Na^+ , 100], 321 [*M* – Boc) H^+]. ^1H NMR (500 MHz, CDCl_3): δ 7.36–7.23 (m, 5H), 4.64 (d, 1H, $J = 6.4$ Hz), 4.53 (d, 1H, $J = 12.2$ Hz), 4.49 (d, 1H, $J = 12.2$ Hz), 4.12 (d, 1H, $J = 9.8$ Hz), 4.03 (d, 1H, $J = 9.8$ Hz), 3.77 (s, 3H), 3.62 (m, 1H), 3.56 (m, 1H), 2.63 (s, 3H), 2.58 (m, 1H), 1.44 (s, 9H). ^{13}C NMR (75 MHz, CDCl_3): δ 174.0, 167.5, 148.7, 137.4, 128.7, 128.1, 127.7, 83.8, 78.7, 74.2, 73.7, 70.8, 60.7, 53.2, 52.4, 44.8, 28.0. Anal. Calcd for $\text{C}_{21}\text{H}_{28}\text{N}_2\text{O}_7$: C, 59.99; H, 6.71; N, 6.66. Found: C, 59.71; H, 6.58; N, 6.27.

(2*R*,3*S*)-1-*tert*-Butyl 2-Methyl 2-[(Benzyloxy)methyl]-3-hydroxy-4-methylene-5-oxopyrrolidine-1,2-dicarboxylate (22**).** Pd(OH)₂ (22.4 mg) was added to a stirred solution of (*3aR*,*6R*,*6aS*)-**20** (165.1 mg, 0.39 mmol) in EtOAc (3.0 mL) placed under N_2 . The mixture was stirred under H_2 at rt for 8 h. The catalyst was filtered through Celite and washed with EtOAc. Evaporation under reduced pressure afforded the crude product as a colorless foam (163.5 mg), which was directly engaged in the following step

without further purification. To a solution of this product in dry THF (3.0 mL) was added under Ar MeI (0.69 mL, 11.0 mmol). The solution was stirred at rt for 24 h. Then MeI (0.69 mL, 11.0 mmol) and NaHCO_3 (66.9 mg, 0.80 mmol) were added to the mixture. The reaction medium was stirred at rt for 3 days. After elimination of the solvent under reduced pressure, the crude product was purified by filtration over silica gel (eluent Et_2O) to give (*2R*,*3S*)-**22** as a clear oil (136.7 mg, 89% for three steps). $[\alpha]_{\text{D}}^{27} -32.6$ (c 1.13, CHCl_3). IR (cm^{-1}): 3448, 2943, 1772, 1746, 1454, 1369, 1304, 1248, 1151, 1116, 1048. MS (ESI, MeCN + CH_2Cl_2 , *m/z*): 414 (MNa^+), 314 [(*M* – Boc) Na^+ , 100]. ^1H NMR (500 MHz, CDCl_3): δ 7.38–7.25 (m, 5H), 6.41 (d, 1H, $J = 2.7$ Hz), 5.83 (d, 1H, $J = 2.1$ Hz), 4.95 (m, 1H), 4.57 (d, 1H, $J = 12.2$ Hz), 4.54 (d, 1H, $J = 12.2$ Hz), 4.10 (d, 1H, $J = 10.0$ Hz), 4.07 (d, 1H, $J = 10.0$ Hz), 3.75 (s, 3H), 2.57 (br m, 1H), 1.47). ^{13}C NMR (75 MHz, CDCl_3): δ 168.9, 165.0, 149.8, 140.6, 137.5, 128.7, 128.1, 127.8, 123.5, 84.3, 73.7, 71.5, 70.3, 69.3, 52.7, 28.0. HRMS (ESI, MeCN + CH_2Cl_2 , *m/z*): calcd for $\text{C}_{20}\text{H}_{25}\text{NO}_7\text{Na}$ (MNa^+) 414.1529, found 414.1536.

(2*R*,3*S*)-Methyl 2-[(Benzyloxy)methyl]-3-hydroxy-4-methylene-5-oxopyrrolidine-2-carboxylate (25**).** To a solution of compound **22** (133.9 mg, 0.34 mmol) in CH_2Cl_2 (1.3 mL) was added TFA (250 μL , 3.37 mmol) at rt, and the mixture was stirred for 50 min. After dilution with CH_2Cl_2 and addition of saturated aqueous Na_2CO_3 , the aqueous phase was extracted with CH_2Cl_2 . The organic phases were dried over MgSO_4 , and the solvent was evaporated to afford (*2R*,*3S*)-**25** (100.1 mg, 100%) as a colorless oil. $[\alpha]_{\text{D}}^{25} -7.0$ (c 1.10, CHCl_3). IR (cm^{-1}): 3290, 2866, 1737, 1694, 1666, 1433, 1314, 1233. MS (ESI, MeOH + CH_2Cl_2 , *m/z*): 605 [(*2MNa*) $^+$, 100], 314 (MNa^+). ^1H NMR (500 MHz, CDCl_3): δ 7.36–7.26 (2 m, 5H), 6.47 (br d, 1H exch., $J = 7.9$ Hz), 6.26 (d, 1H, $J = 2.1$ Hz), 5.75 (br s, 1H), 4.59 (m, 1H), 4.56 (d, 1H, $J = 12.2$ Hz), 4.53 (d, 1H, $J = 12.2$ Hz), 4.01 (d, 1H, $J = 9.1$ Hz), 3.81 (s, 3H), 3.41 (d, 1H, $J = 9.1$ Hz), 2.71 (m, 1H exch.). ^{13}C NMR (75.0 MHz, CDCl_3): δ 170.6, 168.3, 141.2, 137.3, 128.6, 128.0, 127.7, 122.0, 73.7, 73.2, 71.9, 70.8, 53.1. HRMS (ESI, MeOH + CH_2Cl_2 , *m/z*): calcd for $\text{C}_{15}\text{H}_{17}\text{NO}_5\text{Na}$ (MNa^+) 314.1004, found 314.1000.

(2*R*,3*S*)-Methyl 2-[(Benzyloxy)methyl]-3-[(*tert*-butyldimethylsilyl)oxy]-4-methylene-5-oxopyrrolidine-2-carboxylate (26**).** To a stirred solution of **25** (97.5 mg, 0.33 mmol) in CH_2Cl_2 (2.0 mL) were successively added under argon at rt 2,6-lutidine (218 μL , 1.87 mmol) and (TBS)OTf (285 μL , 1.22 mmol). The mixture was stirred at rt for 24 h. Then a solution of Na_2CO_3 (10% w/v) was added until pH 8, and the product was extracted with CH_2Cl_2 . Drying on MgSO_4 and evaporation of the organic phase under reduced pressure afforded (*2R*,*3S*)-**26** as a white solid (131.9 mg, 97%). Mp: 99 °C. $[\alpha]_{\text{D}}^{25} +15.0$ (c 1.28, CHCl_3). IR (cm^{-1}): 3192, 2926, 2854, 1741, 1702, 1674, 1452, 1428, 1359, 1343, 1250, 1235, 1109, 835. MS (ESI, MeOH + CH_2Cl_2 , *m/z*): 428 (MNa^+ , 100). ^1H NMR (500 MHz, CDCl_3): δ 7.38–7.28 (m, 5H), 6.18 (br s, 1H, NH), 6.16 (d, 1H, $J = 2.2$ Hz), 5.51 (br s, 1H), 4.63 (m, 1H, $J = 2.2$ Hz), 4.56 (d, 1H, $J = 12.2$ Hz), 4.54 (d, 1H, $J = 12.2$ Hz), 4.06 (d, 1H, $J = 9.2$ Hz), 3.73 (s, 3H), 3.42 (d, 1H, $J = 9.2$ Hz), 0.87 (s, 9H), 0.10 (s, 3H), 0.09 (s, 3H). ^{13}C NMR (75 MHz, CDCl_3): δ 169.5, 167.9, 141.9, 137.3, 128.6, 128.1, 127.9, 119.4, 73.8, 73.0, 72.2, 69.6, 52.6, 25.7, 17.9, –4.2, –4.6. HRMS (ESI, MeOH + CH_2Cl_2 , *m/z*): calcd for $\text{C}_{21}\text{H}_{31}\text{NO}_5\text{SiNa}$ (MNa^+) 428.1869, found 428.1858.

(2*R*,3*S*)-Methyl 2-[(Benzyloxy)methyl]-3-[(*tert*-butyldimethylsilyl)oxy]-1-(4-methoxybenzyl)-4-methylene-5-oxopyrrolidine-2-carboxylate [(2*R*,3*S*)-18**].** Cs_2CO_3 (151.5 mg, 0.46 mmol) was added to a solution of **26** (123.4 mg, 0.30 mmol) in anhydrous DMF (1.5 mL) under Ar. The mixture was stirred for 10 min at rt and cooled to 0 °C before dropwise addition of (PMB)Br (67.0 μL , 0.46 mmol). The reaction mixture was stirred for 0.5 h at 0 °C and then 16 h at rt. After addition of H_2O and extraction with EtOAc, the organic solution was dried on MgSO_4 , filtered, and evaporated under reduced pressure. The crude product was purified by preparative TLC (eluent heptane–EtOAc, 7:3) to give (*2R*,*3S*)-**18** (146.7 mg, 92%) as a

colorless oil. $[\alpha]_D^{25} +49.2$ (c 1.28, CHCl_3). IR (cm^{-1}): 2950, 2929, 2857, 1740, 1699, 1612, 1512, 1398, 1245, 1125, 836. MS (ESI, $\text{MeOH} + \text{CH}_2\text{Cl}_2$, m/z): 548 $[(\text{MNa})^+, 100]$. ^1H NMR (300 MHz, CDCl_3): δ 7.40–7.25 (m, 5H), 7.10 (d, 2H, $J = 8.7$ Hz), 6.73 (d, 2H, $J = 8.7$ Hz), 6.13 (d, 1H, $J = 2.9$ Hz), 5.40 (d, 1H, $J = 2.6$ Hz), 5.04 (dd, 1H, $J \approx J' \approx 2.7$ Hz), 4.70 (d, 1H, $J = 15.1$ Hz), 4.43 (d, 1H, $J = 11.9$ Hz), 4.32 (d, 1H, $J = 11.9$ Hz), 4.12 (d, 1H, $J = 15.1$ Hz), 3.83 (2 d, 2H), 3.74 (s, 3H), 3.30 (s, 3H), 0.85 (s, 9H), 0.11 (s, 3H), 0.00 (s, 3H). ^{13}C NMR (75 MHz, CDCl_3): δ 169.3, 168.0, 159.0, 142.3, 137.3, 130.5, 128.6, 128.5, 128.4, 128.2, 116.4, 113.6, 73.4, 71.6, 71.1, 65.0, 55.3, 51.8, 43.8, 25.7, 17.9, -4.2, -4.8. HRMS (ESI, $\text{MeOH} + \text{CH}_2\text{Cl}_2$, m/z): calcd for $\text{C}_{29}\text{H}_{39}\text{NO}_6\text{SiNa}$ ($\text{MNa})^+$ 548.2444, found 548.2417.

(2R,3S,4R)-Methyl 2-[(Benzoyloxy)methyl]-3-[(tert-butyltrimethylsilyl)oxy]-1-(4-methoxybenzyl)-4-methyl-5-oxopyrrolidine-2-carboxylate [(2R,3S,4R)-19]. A solution of (2R,3S)-18 (144.3 mg, 0.275 mmol) in EtOAc (5.0 mL) was stirred under H_2 in the presence of PtO_2 (15.5 mg) at rt for 1.5 h. Filtration through Celite and washing with EtOAc afforded the crude product (144.5 mg, 100%), which was purified by preparative thin layer chromatography (eluent heptane–EtOAc, 6:4) to give the diastereomer (2R,3S,4R)-19 (128.4 mg, 89%) as a colorless oil. $[\alpha]_D^{25} +18.5$ (c 1.43, CHCl_3). IR (cm^{-1}): 2948, 2928, 2856, 1738, 1693, 1612, 1512, 1400, 1243, 1076, 832. MS (ESI, $\text{MeOH} + \text{CH}_2\text{Cl}_2$, m/z): 550 $[(\text{MNa})^+, 100]$. ^1H NMR (500 MHz, CDCl_3): δ 7.36–7.30 (m, 3H), 7.21 (d, 2H, $J = 7$ Hz), 7.14 (d, 2H, $J = 8.5$ Hz), 6.75 (d, 2H, $J = 8.5$ Hz), 4.62 (d, 1H, $J_{3,4} = 9.2$ Hz), 4.40 (d, 1H, $J = 15.3$ Hz), 4.32 (d, 1H, $J = 15.3$ Hz), 4.28 (d, 1H, $J = 11.9$ Hz), 4.21 (d, 1H, $J = 11.9$ Hz), 3.77 (masked d, 1H), 3.75 (s, 3H), 3.69 (d, 1H, $J = 10.4$), 3.42 (s, 3H), 2.71 (dq, 1H), 1.22 (d, 3H, $J = 7.6$ Hz), 0.84 (s, 9H), -0.03 (s, 3H), -0.04 (s, 3H). ^{13}C NMR (75.0 MHz, CDCl_3): δ 177.7, 170.0, 158.9, 137.4, 130.2, 129.3, 128.5, 128.1, 128.0, 113.6, 73.9, 73.3, 70.2, 67.2, 55.4, 51.8, 44.2, 40.8, 25.7, 18.0, 11.3, -4.7, -5.1. HRMS (ESI, $\text{MeOH} + \text{CH}_2\text{Cl}_2$, m/z): calcd for $\text{C}_{29}\text{H}_{41}\text{NO}_6\text{SiNa}$ ($\text{MNa})^+$ 550.2601, found 550.2578.

(2R,3S,4R)-Methyl 3-[(tert-Butyldimethylsilyl)oxy]-2-(hydroxymethyl)-1-(4-methoxybenzyl)-4-methyl-5-oxopyrrolidine-2-carboxylate [(2R,3S,4R)-4]. A 14.7 mg portion of 10% Pd/C was added to a solution of (2R,3S,4R)-19 (52.3 mg, 0.099 mmol) in EtOH (0.90 mL) placed under N_2 . Then the mixture was flushed with H_2 gas and stirred under H_2 at rt for 24 h. Filtration of the catalyst through Celite and washing with EtOH afforded pure product (2R,3S,4R)-4 (43.3 mg, 100%) as a colorless oil. $[\alpha]_D^{27} -6.9$ (c 1.23, CHCl_3) [lit. -7.3 (c 0.16, CHCl_3),^{10a} -4.4 (c 2.8, CHCl_3)].¹⁰ⁱ IR (cm^{-1}): 3377, 2930, 2856, 1738, 1669, 1612, 1512, 1436, 1409, 1245, 1177, 1029, 832. MS (ESI, $\text{MeOH} + \text{CH}_2\text{Cl}_2$, m/z): 460 $[(\text{MNa})^+, 100]$. ^1H NMR (500 MHz, CDCl_3): δ 7.29 (d, 2H, $J = 8.4$ Hz), 6.85 (d, 2H, $J = 8.4$ Hz), 5.00 (d, 1H, $J = 15.1$ Hz), 4.56 (d, 1H, $J = 9.1$ Hz), 3.83 (dd, 1H, $J = 12.9$, $J' = 10.1$ Hz), 3.79 (s, 3H), 3.78 (d, 1H, $J = 15.1$ Hz), 3.74 (dd, 1H, $J = 12.9$, $J' = 4.7$ Hz), 3.64 (s, 3H), 2.71 (dq, 1H, $J = 9.1$, $J' = 7.7$ Hz), 1.23 (d, 3H, $J = 7.7$ Hz), 1.15 (dd, 1H, $J = 10.1$, $J' = 4.7$ Hz), 0.88 (s, 9H), 0.08 (s, 3H), 0.07 (s, 3H). ^{13}C NMR (75 MHz, CDCl_3): δ 178.1, 170.6, 159.4, 129.8, 129.7, 114.5, 75.8, 70.0, 61.7, 55.4, 52.2, 44.4, 40.8, 25.8, 18.1, 11.3, -4.7, -5.0. HRMS (ESI, $\text{MeOH} + \text{CH}_2\text{Cl}_2$, m/z): calcd for $\text{C}_{22}\text{H}_{35}\text{NO}_6\text{SiNa}$ ($\text{MNa})^+$ 460.2131, found 460.2137.

Acknowledgment. We are grateful to Prof. G. Pattenden for providing the NMR spectra and $[\alpha]_D$ value of **4** and to ICSN (CNRS, Gif-sur-Yvette) for a grant (J.-C. L.).

Supporting Information Available: Experimental procedures for compounds (**±**)-7, **13**, **14**, **16**, **17**, **18**, **19**, (3aS,6R,6aR)-**21**, (**±**)-**23**, **24**, **27**, and **28** and ^1H and ^{13}C NMR spectra of all compounds. This material is available free of charge via the Internet at <http://pubs.acs.org>.

JO701968D