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A Short, Stereocontrolled, and Practical Synthesis of α-Methylomuralide, a Potent Inhibitor of Proteasome Function

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An efficient and practical synthesis of α-methylomuralide (**3**), a selective inhibitor of proteasomes, has been developed as outlined in Scheme 1. Among the advantages of this route of synthesis over previously described approaches are (1) ease of scale-up and (2) high yields (28% overall yield of α-methylomuralide from **6**) and stereocontrol (including high enantiocontrol). The synthesis is well suited to the production of **3** in the quantities needed for material-intensive in vivo investigations.

Lactacystin (**1**), a microbial product first isolated by Omura[1,2] et al. from a screening project to detect neurotrophic (nerve growth factor-like) activity, was later found to be a highly selective and potent irreversible inactivator of proteasomes.[3,4] The proteasome is a cylindrical, multiprotein assembly which effects the degradation of ubiquitin-tagged proteins. Proteasome action is crucial to the cleavage of a wide variety of proteins including not only misfolded and denatured molecules[5] but also proteins involved in the cell cycle,[6] gene transcription,[7] and cell function. Inactivation of the proteasome by acylation of a critical catalytic threonine subunit is actually mediated by the β-lactone **2**,[4,8,11,13] which we have previously designated as "omuralide"[11] because of its key role in this inactivation. Synthetic **1** and **2**[11−13] have been used in many hundreds of biological laboratories as a reagent to evaluate the role of the proteasome in determining the concentrations and mode of disposal of many proteins.

During the last several years, a new and important chapter of proteasome inhibition has developed from the finding that proteasome inhibitors increase the sensitivity of cancer cells to apoptosis (biochemically regulated cell death) through their effect on the NF-κ B nuclear signaling pathway.[14] Because proteasome inactivation prevents the degradation of I-κ B, the natural inhibitor of NF-κ B, it prevents NF-κ B migration into the nucleus and subsequent transcriptional activation. This results in cell cycle arrest and a decrease in anti-apoptotic protein formation. Increases in pro-apoptotic Bcl-2 and stress kinase JNK proteins ensue which then lead to the release of cytochrome *c* from mitochondria, activation of the Apaf-1-containing aptosome complex, and formation of caspases 3, 7, and 9, direct effectors of apoptosis.[15−20] Various peptidomimetic proteasome inhibitors are currently being evaluated as novel anticancer agents in clinical trials involving hematological malignancies.[16,21,22] The peptidomimetic proteasome inhibitors currently known are generally chemically reactive electrophiles that carry subunits such as aldehydic formyl, α-haloketone, vinyl sulfone, α,β-epoxycarbonyl, boronic acid, and trifluoroacetyl, groups that can interact with many proteins and cause toxicity problems.[23]

Extensive experience in these laboratories with the determination of proteasome inhibitory activity of many structural analogues of lactacystin[11] suggested that

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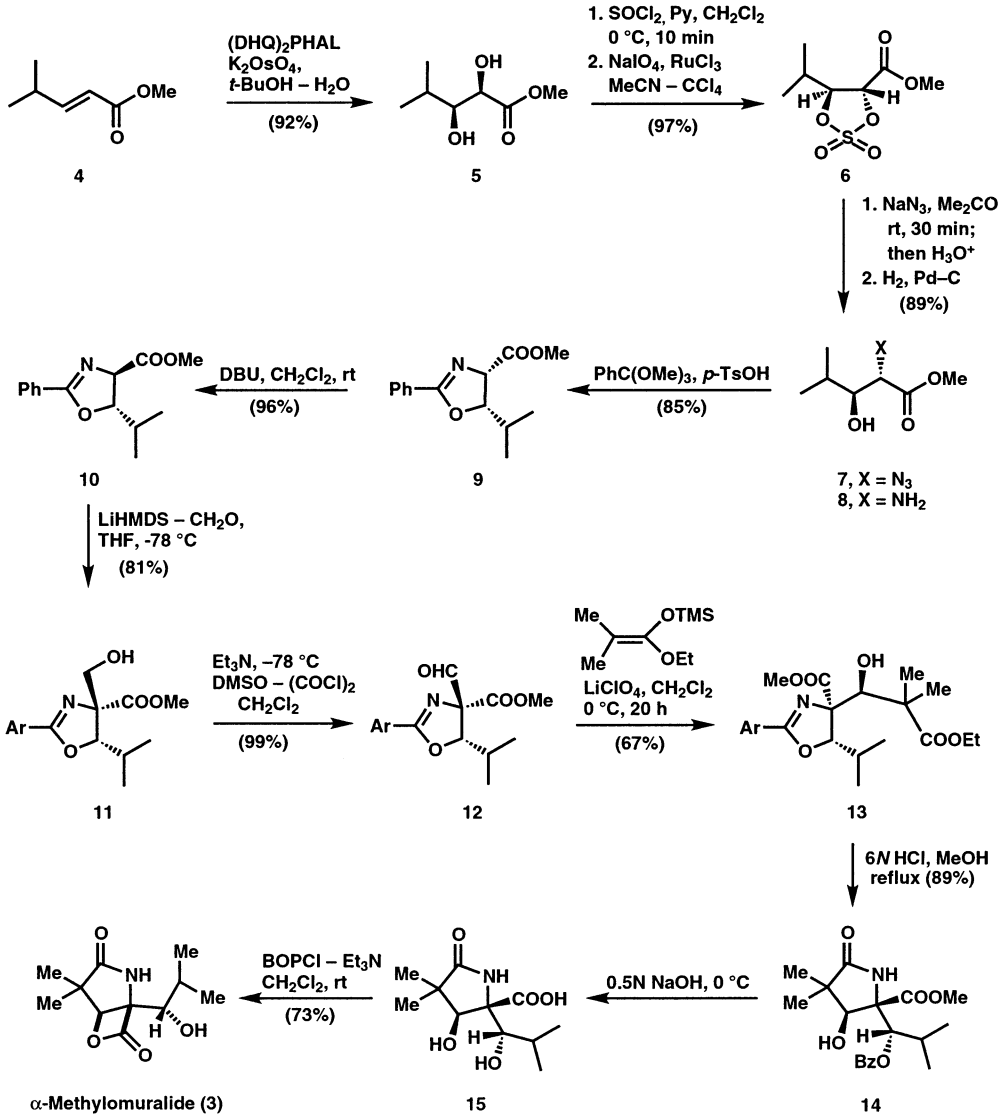
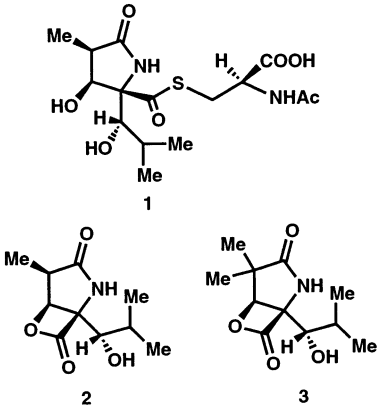
SCHEME 1. Synthesis of α-Methylomuralide (3)



α-methylomuralide (**3**) might be a superior candidate for study as a proteasome-selective anticancer agent. In a previous study, **3** was synthesized and found to be nearly as potent as omuralide (**2**) ($k_{inactivation}$ 2300 M^{-1} s^{-1} for **3** vs 3060 M^{-1} s^{-1} for **2** as irreversible inhibitors of the mammalian proteasome).[24] In addition, **3** has the advantage over **2** of superior hydrolytic stability. We therefore have developed an improved synthesis of **3** that is enantio- and diastereoselective, short, and easily executed on large scale. The pathway of synthesis is summarized in Scheme 1.



The *E*-ester **4**[25] was subjected to Sharpless asymmetric dihydroxylation[26] at 5 °C for 3 days with a catalytic amount of $(DHQ)_2PHAL$ ligand (1 mol %, Aldrich) and $K_2OsO_2(OH)_4$ (0.5 mol %) and stoichiometric $K_3Fe(CN)_6$ in 1:1 *t*-BuOH−H_2O containing $CH_3SO_2NH_2$ to give after recrystallization from EtOAc enantiomerically pure crystalline diol **5** in 92% yield, mp 33−35 °C,[27] $[\alpha]^{23}_D$ −4.8 (*c* = 2.7, $CHCl_3$). Cyclic sulfite formation from **5** and subsequent oxidation with catalytic RuO_4 and stoichiometric $NaIO_4$[28] provided the crystalline cyclic sulfate **6**,

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mp 63−65 °C, $[\alpha]^{23}_D$ −22.8 (c = 1.0, $CHCl_3$). Selective displacement of sulfate at C(α) of **6** by NaN_3 in 4:1 acetone−water generated the corresponding α-azido β-monosulfate ester which was stirred with ether−2 N H_2SO_4 at 23 °C for 16 h to form the oily azido alcohol **7**, $[\alpha]^{23}_D$ −47.6 (c = 1.1, $CHCl_3$) in 90% yield. Catalytic hydrogenation of **7** over Pd−C at 23 °C and 1 atm H_2 for 24 h provided in 99% yield (2*S*,3*S*)-3-hydroxyleucine methyl ester **8**, $[\alpha]^{23}_D$ +8.6 (c = 0.7, $CHCl_3$). Treatment of **8** with trimethylorthobenzoate (3 equiv) and *p*-toluenesulfonic acid (1 equiv) in dimethoxyethane at reflux for 4 h[29] gave the oily *cis*-oxazoline **9**, $[\alpha]^{23}_D$ −74.4 (c = 1.8 $CHCl_3$), in 85% yield. Exposure of **9** to 5 mol % of 1,8-diazobicyclo[5.4.0]undec-7-ene at 23 °C for 12 h effected complete isomerization to the more stable *trans*-oxazoline **10**, $[\alpha]^{23}_D$ −107 (c = 3.0, $CHCl_3$) (96% yield).

Deprotonation of **10** in THF at −78 °C with lithium hexamethyldisilazane for 1 h and treatment with ethereal formaldehyde[30] at −78 °C for 3 h produced the α-hydroxymethylated ester oxazoline **11** diastereoselectively in 81% yield, mp 76−78 °C, $[\alpha]^{23}_D$ −4.4 (c = 1 in $CHCl_3$). Swern oxidation of **11** (2 equiv of ClCOCOCl, 3 equiv of dimethyl sulfoxide reagent preformed in CH_2Cl_2 at −78 °C for 30 min and added via cannula to a mixture of **11** and excess Et_3N in CH_2Cl_2 at −78 °C followed by addition of pentane, filtration, and nonaqueous isolation) gave the sensitive aldehyde **12** in 99% yield.[31] Mukaiyama aldol coupling[32] of **12** with the TMS enol ether of ethyl isobutyrate in CH_2Cl_2 at 0 °C in the presence of lithium perchlorate (1.1 equiv)[33] at −20 °C for 2 h afforded in 67% yield and diastereoselectively the required isomer **13**, $[\alpha]^{23}_D$ −18.2 (c = 1.8, $CHCl_3$). Heating of **13** in 1:1 6 N HCl−CH_3OH at reflux for 5 h produced in 89% yield the γ-lactam **14**, $[\alpha]^{23}_D$ +25.7 (c = 3.0 in $CHCl_3$). Saponification of **14** in 0.5 N aqueous NaOH at 5 °C for 40 h gave the dihydroxy acid **15**, which was transformed directly into α-methylomuralide **3** (73% from **14**) by reaction with bis(2-oxo-3-oxazolidinyl)phosphinic chloride (BOPCl) and Et_3N in CH_2Cl_2 at 23 °C. Spectral and physical data for **3**, $[\alpha]^{23}_D$ −84.6 (c = 1.0, EtOAc), mp 181−183 °C, matched those of an authentic sample.[24,34]

In summary, the simple, enantio- and diastereocontrolled synthesis of α-methylomuralide (**3**) described herein provides access to this stable and potent analogue of omuralide in quantity. We believe that further biological studies with **3** will be facilitated by its ready availability, especially for more material-intensive in vivo investigations, and that **3** will allow a more rapid and complete examination of the therapeutic possibilities of highly selective proteasome inhibition.

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(31) The aldehyde **12** is rapidly deformylated to form **10** upon exposure to H_2O or silica gel.
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(33) As Lewis acid catalyst for the conversion of **12** to **13**, $LiClO_4$ in CH_2Cl_2 was far superior to $SnCl_4$, $TiCl_4$, $ZnCl_2$, $Cu(OTf)_2$, or MgI_2. The face selectivity of coupling at the formyl center of **12** is readily explained in terms of a bidentate coordination of the formyl and methoxycarbonyl oxygens with lithium.
(34) Except for the labile aldehyde **12**, all synthetic intermediates were readily purified by column chromatography on silica gel or by recrystallization (in the case of solids). Satisfactory spectroscopic and high-resolution mass spectral data were obtained for each reaction product.

Experimental Section

(2*R*,3*S*)-2,3-Dihydroxy-4-methylpentanoic Acid Methyl Ester, 5. A solution of $(DHQ)_2PHAL$ (1.76 g, 2.24 mmol), $K_2OsO_2(OH)_4$ (412 mg, 1.12 mmol), $K_3[Fe(CN)_6]$ (220 g, 0.67 mol), K_2CO_3 (92 g, 0.67 mol), $NaHCO_3$ (56 g, 0.67 mol), and $MeSO_2NH_2$ (21.3 g, 0.224 mol) in *t*-BuOH−H_2O (1:1, 2 L) was stirred at room temperature for 10 min. The resulting orange homogeneous solution was cooled to 0 °C and treated with (*E*)-4-methylpent-2-enoic methyl ester (28.8 g, 0.224 mol). The mixture was stirred vigorously for 3 days at 5 °C using a mechanical stirrer. After completion, the reaction mixture was treated with $Na_2S_2O_5$ (180 g, 1.0 mol), and stirring was continued for 1 h at room temperature. The *t*-BuOH layer was separated and kept aside; the aqueous layer was extracted with EtOAc (4 × 250 mL). The combined organic extracts were washed with 1 N NaOH (300 mL) and brine (300 mL), dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by a short silica gel column (using eluent 50−100% EtOAc) and further recrystallized in hot EtOAc (∼100 mL) to give pure diol **5** as white solid (33 g, 92%). Data for **5**: mp 33−35 °C; R_f 0.50 (Hex−EtOAc 50:50); $[\alpha]^{23}_D$ −4.8 (c 2.7, $CHCl_3$); FTIR (film) ν_{max} 3456, 2954, 1746, 1390, 1256, 1049 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 4.23 (1H, d, J = 7.2 Hz), 3.75 (3H, s), 3.43 (1H, dd, J = 8.6, 1.2 Hz), 3.19 (1H, d, J = 5.6 Hz), 1.80 (1H, m), 0.98 (3H, d, J = 6.8 Hz), 0.91 (3H, d, J = 6.5 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 174.8, 78.1, 71.6, 53.0, 31.3, 19.3, 19.2; LRMS (m/z)162.9 $(M + H)^+$ (100); EIMS m/z calcd for $C_7H_{14}O_4$ 162.1837, found 162.1832.

Cyclic Sulfate of (2*R*,3*S*)-2,3-Dihydroxy-4-methylpentanoic Acid Methyl Ester, 6. To an ice-cold solution of diol **5** (30.8 g, 0.19 mol) in CH_2Cl_2 (400 mL) was added pyridine (62 mL, 0.76 mol) and the mixture stirred for 5 min. $SOCl_2$ (20.5 mL, 0.285 mol) was added to the above solution dropwise over a period of 15 min, maintaining the temperature below 5 °C. Stirring was continued for another 30 min at 0 °C, and the mixture was diluted with a cold ether−water (1:1, 400 mL) mixture. The aqueous layer was separated and extracted with ether (2 × 200 mL), and the combined organic layers were washed with cold water and brine. The organic phase was dried (Na_2SO_4) and condensed under reduced pressure to give the crude sulfite (crude ∼39.5 g) as colorless oil in quantitative yield. Thus obtained cyclic sulfite analytically was pure enough for the next step. The crude cyclic sulfite was dissolved in a mixture of CCl_4−MeCN (1:1, 180 mL) and treated with $NaIO_4$ (60.8 g, 0.28 mol) followed by $RuCl_3$ (59 mg, 0.28 mmol) and water (240 mL). The resulting biphasic reaction medium was stirred vigorously for 90 min at room temperature. The mixture was diluted with ether (250 mL), and the aqueous layer was separated and extracted with ether (2 × 250 mL). The combined organic layers were washed with water, saturated $NaHCO_3$, and brine and dried over Na_2SO_4. The solution was filtered and concentrated in vacuo to give cyclic sulfate **6** (41.5 g, 97%) as a colorless solid upon cooling. The crude material was pure enough for further reaction, although a small amount of **6** (0.5 g) was purified by silica gel column (50% EtOAc as eluent) for analytical purposes. Data for **6**: mp 63−65 °C; R_f 0.39 (Hex−EtOAc 50:50); $[\alpha]^{23}_D$ −22.8 (c 1.0, $CHCl_3$); FTIR (film) ν_{max} 2974, 1746, 1473, 1216, 1018 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 4.89 (1H, d, J = 6.5 Hz), 4.73 (1H, apparent t, J = 6.2 Hz), 3.83 (3H, s), 2.16 (1H, m), 1.05 (3H, d, J = 6.0 Hz), 1.02 (3H, d, J = 6.5 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 166.0, 88.4, 78.1, 53.9, 31.2, 17.9, 16.8; LRMS (m/z) 242.3 $(M + NH_4)^+$ (50), 223.0 (20), 209.1 (100); EIMS m/z calcd for $C_7H_{16}NO_6S$ 242.2712, found for $(M + NH4)^+$ 242.2714.

(2*S*,3*S*)-2-Azido-3-hydroxy-4-methylpentanoic Acid Methyl Ester, 7. A solution of cyclic sulfate **6** (41.0 g, 0.18 mol) in acetone−water (5:1, 600 mL) was treated with NaN_3 (21.9 g, 0.36 mol) at room temperature and stirred vigorously for 2 h. After completion, the reaction mixture was concentrated (150 mL) in vacuo, diluted with ether−2 N H_2SO_4 (1:1, 1 L), and allowed to stir at room temperature overnight to give

the crude azido alcohol **7** (31.2 g, 90%) as a viscous oil. The crude product was pure enough for next step. A small amount of azido alcohol was purified by silica gel column to give analytically pure **7** as a colorless oil. Data for **7**: R_f 0.75 (Hex−EtOAc 50:50); $[\alpha]^{23}_D$ −47.6 (*c* 1.1, $CHCl_3$); FTIR (film) ν_{max} 3498, 2964, 2118, 1744, 1434, 1513, 1256, 980 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 3.90 (1H, d, J = 6.4 Hz), 3.80 (3H, s), 3.65 (1H, q, J = 6.2 Hz), 2.56 (1H, br s, −*OH*), 1.89 (1H, m), 0.97 (3H, d, J = 6.8 Hz), 0.95 (3H, d, J = 6.5 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 170.3, 76.6, 64.0, 52.9, 30.4, 19.5, 16.7; LRMS (*m*/*z*) 210.0 (70) $(M + Na)^+$, 165.2 (100), 129.4 (40); EIMS *m*/*z* calcd for $C_7H_{14}N_3O_3$ 188.2044, found $(M + H)^+$ 188.2039.

(2*S*,3*S*)-3-Hydroxyleucine Methyl Ester, 8. A solution of azido alcohol **7** (24 g, 0.13 mol) in 20% EtOH−EtOAc (0.5 L) at room temperature was treated with 10% Pd−C (5 g) under an argon atmosphere. The reaction setup was kept under vacuum and purged with H_2 gas (four times); the resulting dark suspension was stirred vigorously in an atmosphere of H_2 (1 atm, H_2 balloon). After 24 h, the reaction mixture was filtered through Celite and concentrated in vacuo to give the crude amino alcohol **8** (20.8 g, 99%) as a yellow viscous oil and subjected to the next step without further purification. Data for **8**: R_f 0.10 (neat EtOAc); $[\alpha]^{23}_D$ +8.6 (*c* 0.7, $CHCl_3$); FTIR (film) ν_{max} 3494, 2898, 1738, 1513, 1252, 988 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 4.10 (1H, m), 3.79 (3H, s), 3.69 (1H, d, J = 6.5 Hz), 3.01 (3H, bs), 1.72 (1H, m), 0.98 (3H, d, J = 4.6 Hz), 0.92 (3H, d, J = 4.2 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 175.1, 80.1, 57.6, 52.1, 31.5, 20.4, 18.2; LRMS (*m*/*z*) 162.1 (40) $(M + H^+)$, 128.4 (50), 112.1 (30); EIMS *m*/*z* calcd for $C_7H_{15}NO_3$ 161.1989, found 161.1987.

Methyl (4*S*,5*S*)-5-Isopropyl-2-phenyl-4,5-dihydrooxazole-4-carboxylate, 9. To a mixture of (2*S*,3*S*)-3-hydroxyleucine methyl ester (16.7 g, 0.1 mol) and *p*-TsOH·H_2O (19.0 g, 0.1 mol) in dimethoxyethane (250 mL) was added trimethyl orthobenzoate (53.7 mL, 0.313 mol), and the mixture was refluxed for 4 h. After completion, the reaction mixture was diluted with water (200 mL), and the aqueous layer was separated and extracted with ether (3 × 150 mL). The combined organic layers were washed with water and brine and dried over Na_2SO_4. The solvent was removed in vacuo to give crude oxazoline **9** and excess orthobenzoate. Flash column chromatography on silica gel column (eluent 10−60% EtOAc−hexane) afforded the pure oxazoline **9** (21.8 g, 85%) as viscous oil. Data for **9**: R_f 0.55 (Hex−EtOAc 50:50); $[\alpha]^{23}_D$ −74.4 (*c* 1.8, $CHCl_3$); FTIR (film) ν_{max} 2950, 1746, 1544, 1358, 1186, 1010 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.92 (2H, d, J = 8.9 Hz), 7.39 (1H, m), 7.34 (2H, m), 4.87 (1H, d, J = 8.9 Hz), 4.45 (1H, dd, J = 10.0, 8.0 Hz), 3.67 (3H, s), 1.94 (1H, m), 0.96 (3H, d, J = 6.5 Hz), 0.93 (3H, d, J = 6.4 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 170.7, 166.8, 131.9, 128.7, 128.4, 127.4, 87.7, 70.6, 52.3, 29.4, 19.8, 18.9; LRMS (*m*/*z*) 248.1 $(M + H^+)$ (100), 171.1 (90), 158.3 (20); EIMS *m*/*z* calcd for $C_{14}H_{17}NO_3$ 247.2897, found 247.2892.

Methyl (4*R*,5*S*)-5-Isopropyl-2-phenyl-4,5-dihydrooxazole-4-carboxylate, 10. A solution of oxazoline **9** (11.8 g, 48.0 mmol) in CH_2Cl_2 (100 mL) was treated with 1,8-diazabicyclo[5.4.0]undec-7-ene (360 μL, 2.4 mmol) at room temperature. After 12 h, the reaction mixture was diluted with ether (250 mL) and washed with water (3×) and brine. The organic layer was dried over Na_2SO_4, and removal of the solvent in vacuo afforded the crude *trans*-oxazoline **10** (11.4 g, 96%) as a yellow viscous oil. A small amount of compound was purified by flash column chromatography (eluent 10−60% EtOAc−hexane) to give pure compound for analytical purposes. Data for **10**: R_f 0.50 (Hex−EtOAc 50:50); $[\alpha]^{23}_D$ −106.8 (*c* 3.0, $CHCl_3$); FTIR (film) ν_{max} 2964, 1746, 1644, 1256, 938 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.98 (2H, d, J = 6.8 Hz), 7.47 (1H, m), 7.39 (2H, m), 4.66 (1H, apparent t, J = 6.4 Hz), 4.56 (1H, d, J = 6.8 Hz), 3.78 (3H, s), 1.94 (1H, m), 1.02 (3H, d, J = 6.8 Hz), 0.98 (3H, d, J = 6.5 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 172.3, 165.9, 131.9, 128.7, 128.5, 127.4, 87.3, 71.4, 52.8, 32.6, 17.5, 17.4; LRMS (*m*/*z*) 248.1 (100) $(M + H^+)$,178.2 (50); EIMS *m*/*z* calcd for $C_{14}H_{17}NO_3$ 247.2897, found 247.2896.

Conversion of *trans*-(4*S*, 5*R*)-Phenyloxazoline to Primary Alcohol 11. To a flame-dried flask was added LiHMDS (1.5 m.mol, 2.27 mL, 0.66 M stock solution in THF), which was then then cooled to −78 °C, immersed in THF (10 mL), and treated dropwise with a solution of oxazoline **10** (4.0 g, 16.2 mmol) in THF (20 mL) to give the yellow transparent solution. Stirring was continued for another 1 h. After the enolate formation was complete, it was subjected to formylation by adding freshly prepared formaldehyde (160 mL, 1 M stock solution in ether, through pre cooled cannula with dry ice)[32] solution at −78 °C, and stirring was continued for 3 h. The mixture was rapidly quenched with water (20 mL) and warmed to room temperature. The organic phase was separated, the aqueous layer was extracted with EtOAc (3 × 200 mL),and the combined organic layers were dried over Na_2SO_4 and concentrated in vacuo. The pure alcohol **11** (3.59 g, 81%) was obtained as a white powder by flash column chromatography using Hex−EtOAc (25% neat EtOAc in hexane) as eluent: mp 76−78 °C; R_f 0.18 (Hex−EtOAc 50:50); $[\alpha]^{23}_D$ −4.4 (*c* 1.0, $CHCl_3$); FTIR (film) ν_{max} 3356, 2978, 1742, 1628, 1498, 1253, 1020, 865 cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz) δ 7.84 (2H, d, J = 8.4 Hz), 7.41 (1H, m), 7.32 (2H, m), 4.40 (1H, d, J = 7.2 Hz), 3.97 (1H, dd, J = 12.0, 6.6 Hz), 3.79 (1H, dd, J = 11.8, 6.4 Hz), 3.69 (3H, s), 3.10 (1H, t, 6.5 Hz), 1.97 (1H, m), 0.95 (3H, d, J = 6.6 Hz), 0.94 (3H, d, J = 6.5 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 171.6, 166.6, 132.0, 128.7, 128.5, 127.1, 89.3, 80.4, 67.0, 52.6, 29.8, 19.7, 18.9; LRMS (*m*/*z*) 277.0 (100) (M^+), 250.1 (60), 178.2 (35), 165.2 (25); EIMS *m*/*z* calcd for $C_{15}H_{19}NO_4$ 277.3157, found 277.3152.

Conversion of Primary Alcohol 11 to Aldehyde 12 (Modified Swern Oxidation). To a solution of oxalyl chloride (875 μL, 10.0 mmol) in CH_2Cl_2 (50 mL) was added dropwise a solution of dry DMSO (1.0 mL, 15.0 mmol) in CH_2Cl_2 (30 mL) at −78 °C. Stirring was continued for 30 min at this temperature. The above preformed Swern reagent was carefully transferred through a cannula (wrapped with dry ice aluminum foil) to a solution containing the mixture of alcohol **11** (1.38 g, 5.0 mmol) and excess Et_3N (3.45 mL, 25.0 mmol) at −78 °C. After 3 h of vigorous stirring, the excess solvent was pumped off in vacuo and the reaction mixture was diluted with pentane (30 mL). The resulting white crystalline Et_3N·HCl salt was filtered off under argon through a sintered funnel fitted with a dry round-bottom flask and nitrogen inlet. The flask was washed with pentane portionwise (3 × 30 mL) and the supernatant solution decanted through a syringe and filtered. The combined filtrates were condensed in vacuo under argon atmosphere (vacuum pump 2 mmHg at rt) to give the aldehyde **12** (pure by ^1H NMR analysis) 1.37 g (99%): R_f 0.28−0.35 (Hex−EtOAc 50:50, decomposes on TLC); $[\alpha]^{23}_D$ −3.4 (*c* 1.7, $CHCl_3$); FTIR (film) ν_{max} 2943, 2860, 1728, 1608, 1416, 1228, cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 9.71 (1H, s), 7.91 (2H, d, J = 8.1 Hz), 7.42 (1H, m), 7.34 (2H, m), 4.84 (1H, d, J = 8.4 Hz), 3.70 (3H, s), 2.01 (1H, m), 1.03 (3H, d, J = 6.4 Hz), 0.87 (3H, d, J = 6.6 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 192.2, 168.3, 167.3, 132.5, 128.8, 128.5, 126.9, 86.8, 85.3, 53.0, 41.0, 29.4, 19.2, 19.1; LRMS (*m*/*z*) 276.1 (45) (M^+), 249.1 (20), 220.2 (100) (45), 171.1 (20), 133.3 (35).

Conversion of Aldehyde 12 to Aldol Product 13. A solution of $LiClO_4$ (580 mg, 5.5 mmol) in CH_2Cl_2 (20 mL) was treated with freshly prepared aldehyde **12** (1.37 g, 5.0 mmol) at 0 °C, and the dimethyl ketene acetal (10 mL, 1 M solution in CH_2Cl_2, 10.0 mmol) was added dropwise at this temperature. After 5 h, the reaction mixture was quenched with saturated $NaHCO_3$ (20 mL), the aqueous layer was extracted with CH_2Cl_2 (3 × 25 mL), and the combined organic layers were washed with water and brine and dried over Na_2SO_4. The crude product was obtained by solvent removal and purified by silica gel column to give the pure aldol product **13** (1.3 g, 67%) as a single diastereomer. Data for **13**: R_f 0.50 (Hex−EtOAc 60:40); $[\alpha]^{23}_D$ −18.2 (*c* 1.8, $CHCl_3$); FTIR (film)

ν_{max} 2954, 2898, 1746, 1644, 1513, 1256, 838 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.78 (2H, d, J = 7.8 Hz), 7.40 (1H, m), 7.31 (2H, m), 4.71 (1H, d, J = 3.8 Hz), 4.50 (1H, d, J = 9.8 Hz), 3.98 (1H, d, J = 10.2 Hz), 3.82 (1H, m), 3.68 (3H, s), 3.60 (1H, m), 1.82 (1H, m), 1.18 (3H, s), 1.06 (3H, s), 1.02 (3H, d, J = 6.7 Hz), 0.84 (3H, t, J = 6.3 Hz), 0.71 (3H, d, J = 6.5 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 176.08, 174.7, 165.3, 132.1, 128.8, 128.5, 127.2, 92.9, 82.4, 80.5, 60.6, 52.9, 46.9, 30.0, 24.8, 21.5, 19.3, 16.1, 13.9; LRMS (m/z) 392.1 (100) ($M + H^+$), 259.4 (20), 226.1 (30), 197.7 (90); EIMS m/z calcd for $C_{21}H_{30}NO_6$ 392.2073, found 392.2068 $(M + H)^+$.

Conversion of Aldol Product 13 to Lactam 14. The aldol product **13** (1.3 g,) was suspended in MeOH−6 N HCl (1:1, 30 mL) and refluxed for 5 h. The mixture was concentrated under reduced pressure and diluted with water (20 mL). The resulting aqueous phase was basified (pH ∼9.0) with solid $NaHCO_3$. The aqueous layer was extracted with EtOAc (3 × 50 mL), and combined organic layers were washed with water and brine and dried (Na_2SO_4). The residue was purified by flash column chromatography to give pure aldol products **14** (1.06 g, 89%) as a yellow semisolid. Data for **14**: R_f 0.38 (Hex−EtOAc 20:80); $[\alpha]^{23}_D$ +25.7 (c 3.0, $CHCl_3$); FTIR (film) ν_{max} 3445, 2938, 1740, 1725, 1710, 1240 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.98 (2H, d, J = 7.9 Hz), 7.51 (1H, m), 7.38 (2H, m), 6.97 (1H, bs, −*NH*), 5.61 (1H, d, J = 5.2 Hz), 3.99 (1H, s), 3.74 (3H, s), 2.12 (1H, m), 0.99 (3H, s), 0.98 (3H, s), 0.94 (3H, d, J = 3.5 Hz), 0.93 (3H, d, J = 3.8 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 182.0, 171.9, 166.6, 133.9, 130.1, 129.2, 128.9, 79.0, 77.9, 70.3, 53.0, 43.6, 30.6, 24.7, 20.8, 18.9, 18.6; LRMS (m/z) 363.6 (30) $(M + H)^+$, 330.8 (100), 278.1 (50), 266.2 (30), 247.9 (20); EIMS m/z calcd for $C_{19}H_{25}NO_6$ 363.4049, found 363.4091.

Conversion of Lactam 14 to α-Methylomuralide (3). A solution of lactam **14** (1.0 g, 2.6 mmol) in 0.5 N NaOH (20 mL) was allowed to stir at 5 °C for 40 h. After hydrolysis was complete, the reaction mixture was acidified with 1 N HCl (30 mL). The aqueous layer was washed with EtOAc (25 mL), separated, and concentrated in vacuo to give the crude carboxylic acid. The crude material was triturated with 10% Et_3N in CH_2Cl_2 (100 mL), filtered through plug of cotton to remove any trance amount of NaCl precipitated, and washed with CH_2Cl_2 (10 mL). The filtrates were concentrated to give the crude acid **15** as a yellow solid. The crude acid was suspended in dry CH_2Cl_2 (10 mL), treated with Et_3N (830 μL, 6.0 mmol), and stirred vigorously at room temperature for 5 min. To this solution was added BOPCl (560 mg, 2.2 mmol) at room temperature under argon, and stirring was continued for 35 min. After completion (TLC), the reaction mixture was quenched with wet EtOAc (5 mL) and stirred for 5 min. The mixture was diluted with water (10 mL), and the aqueous layer was extracted with EtOAc (3 × 20 mL). The combined organic layers were washed with water and filtered through a short pad of silica gel and the silica pad washed repeatedly to give the crude β-lactone. Purification by flash column afforded the pure lactone **3** (336 mg, 73%) as a white solid. Data for **3**: mp 181−183 °C; R_f 0.50 (Hex−EtOAc 20:80); $[\alpha]^{23}_D$ −84.6 (c 1.0, EtOAc); FTIR (film) ν_{max} 3450, 2954, 2890, 1836, 1720, 1640, 1513, 1248 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 6.97 (1H, br s, −*NH*), 4.82 (1H, s), 3.74 (1H, t, J = 3.8 Hz), 3.59 (1H, d, J = 7.6 Hz), 1.81 (1H, m), 1.20 (6H, s), 1.06 (3H, d, J = 6.5 Hz), 0.99 (3H, d, J = 6.7 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 180.2, 170.9, 81.9, 79.4, 72.8, 42.8, 30.8, 23.6, 20.0, 18.7, 17.6; ^1H NMR (Py-d_5, 400 MHz) δ 10.41 (1H, br s, −*NH*), 7.66 (1H, br s, −OH), 5.17 (1H, s), 4.05 (1H, t, J = 7.8 Hz), 1.37 and 1.38 (3H, each s), 1.19 (3H, d, J = 6.5 Hz), 1.17 (3H, d, J = 6.7 Hz); ^{13}C NMR (Py-d_5, 100 MHz) δ 180.8, 171.0, 82.0, 80.2, 72.5, 42.8, 30.9, 23.8, 20.5, 19.1, 17.9; LRMS (m/z) 244.1 $(M + NH_3)^+$ (70), 226.1 (40), 154.3 (20); EIMS m/z calcd for $C_{11}H_{21}N_2O_4$ 245.1501, found for $(M + NH_4)^+$ 245.1508.

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Note Added after ASAP Posting. In the text located above the summary, the physical data for compund **3** was incorrect in the version posted February 20, 2003. The corrected version was posted February 25, 2003.

Supporting Information Available: ^{13}C NMR spectra available for **6**−**13** and **3**. This material is available free of charge via the Internet at http://pubs.acs.org.

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