A Novel Enantioselective Synthetic Route to Omuralide Analogs with the Potential for Species Selectivity in Proteasome Inhibition.

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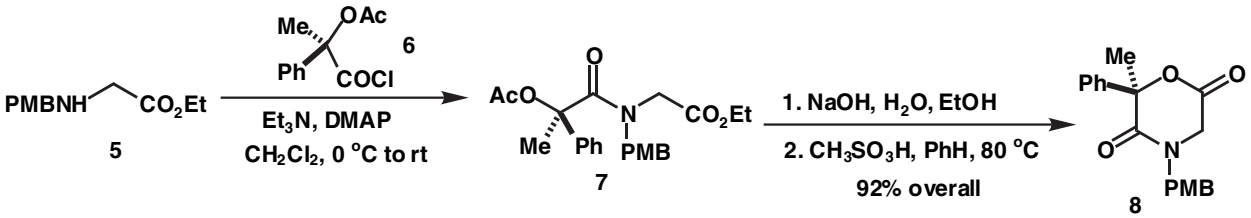
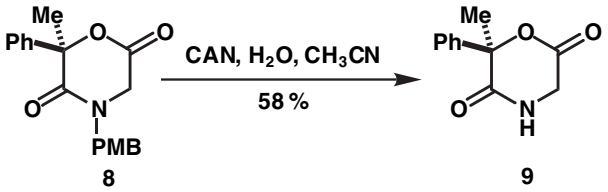
Supporting Information

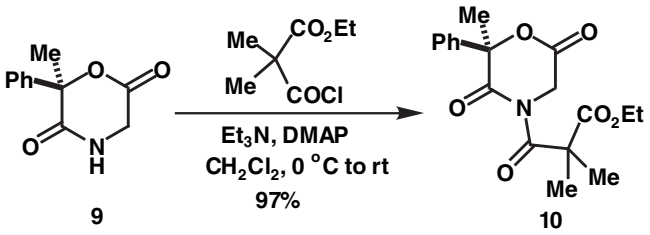


(*6R*)-4-(4'-Methoxybenzyl)-6-methyl-6-phenylmorpholine-2,5-dione (8). (*R*)-Atrolactic acid (6.70 g, 40.3 mmol) was stirred in acetyl chloride (30 mL) at rt for 1.5 h. The excess acetyl chloride was removed by distillation under reduced pressure (50 mm Hg) to afford the crude acetate as a yellow syrup. This material was dissolved in benzene (30 mL) containing DMF (150 μL, 1.92 mmol, 3 mol %) and treated dropwise with oxalyl chloride (11.0 mL, 130 mmol) at rt. The resulting solution was stirred overnight (13 h) and concentrated under reduced pressure (50 mm Hg; oil bath temperature of 30-35°C). Removal of the remaining benzene and oxalyl chloride by the application of high vacuum (0.5 mm Hg) provided the crude acid chloride **6** as a brown syrup that was dissolved in CH_2Cl_2 (20 mL) and added dropwise over 15 min to a 0 °C solution of ethyl *N*-(4-methoxybenzyl)glycinate **5** (7.11 g, 31.9 mmol), 4-(dimethylamino)pyridine (294 mg, 2.41 mmol), and pyridine (5.5 mL, 68 mmol) in CH_2Cl_2 (30 mL). The resulting dark brown solution was stirred at 0 °C for 15 min, then

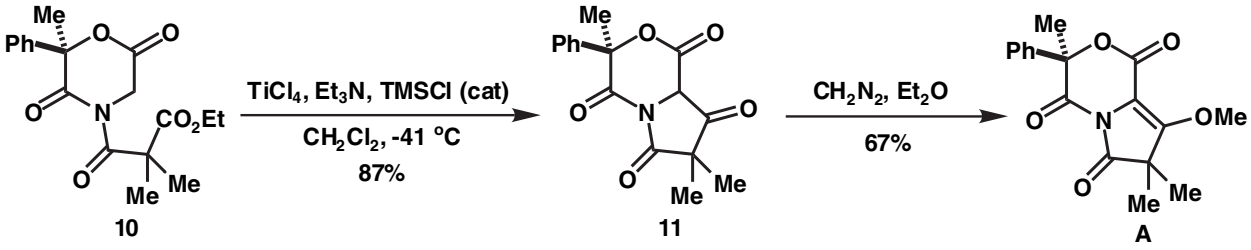
at rt for 4 h and washed with 1 M HCl (2 x 80 mL). The combined aqueous layers were extracted with CH_2Cl_2 (4 x 50 mL). The organics were combined, washed with water (100 mL) and half saturated sodium bicarbonate solution (2 x 100 ml), and dried ($MgSO_4$). Concentration in vacuo afforded the crude ethyl *N*-(*O*-acetylatrolactyl)glycinate **7** as a brown syrup that was dissolved in ethanol (55 mL) and treated with a 2 M solution of NaOH (110 mL) in water with stirring for 24 h at rt. The mixture was adjusted to pH 2 - 3 with 1 M HCl and extracted with ethyl acetate (4 x 70 ml). The organic layers were combined, dried ($MgSO_4$) and concentrated to provide a tan syrup that was dissolved in benzene (100 mL) containing methanesulfonic acid (210 μL, 3.30 mmol, 8 mol %). The mixture was heated at 80 °C for 30 min, cooled to rt and washed with saturated sodium bicarbonate solution (2 x 100 mL). The bicarbonate washings were extracted with ethyl acetate (4 x 70 ml) and the combined organics were washed with saturated sodium chloride solution (130 mL) and dried ($MgSO_4$). Concentration in vacuo provided **8** as a tan syrup (9.54 g, 92 % from N-(4-methoxybenzyl)glycinate). Chromatography (acetone / hexanes (30 / 70)) of a portion gave an analytical sample of **8** as a colorless syrup; $[\alpha]_D^{23}$ -21° (c = 1.4, $CHCl_3$); FTIR 1765, 1678, 1612, 1585, 1513 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.42 - 7.30 (5H, m), 7.10 (2H, d, J = 8.6 Hz), 6.82 (2H, d, J = 8.6 Hz), 4.73 (1H, d, J = 15 Hz), 4.41 (1H, d, J = 15 Hz), 3.80 (1H, d, J = 18 Hz), 3.78 (3H, s), 3.47 (1H, d, J = 18 Hz), 1.90 (3H, s); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 166.8, 166.3, 159.4, 138.8, 129.4, 129.0, 128.7, 126.6, 124.0, 114.2, 85.1, 55.3, 49.3, 48.0, 27.4; EIMS *m/z* calcd. for $C_{19}H_{19}NO_4$ 325.1313, found 325.1314.



(*6R*)-6-Methyl-6-phenylmorpholine-2,5-dione (9). Ceric ammonium nitrate (52.5 g, 95.8 mmol) was added to a solution of the PMB-protected morpholine-2,5-dione **8** (7.75 g, 23.8 mmol) in H_2O (180 mL) and acetonitrile (450 mL). The mixture was stirred at rt for 2.5 h, diluted with water (200 mL) and extracted with ethyl acetate (5 x 200 mL). The organic layers were combined, washed with saturated sodium chloride solution (500 mL) and dried ($MgSO_4$). The solvent was evaporated and the residue redissolved in a mixture of acetonitrile (25 mL), 0.12 M NaH_2PO_4 buffer (25 mL) and 30% aqueous H_2O_2 (1.5 mL). Technical grade (80 % purity) $NaClO_2$ (2.01 g, 17.7 mmol) was added in five portions over 15 min with vigorous stirring at rt for 1.5 h. The acetonitrile was removed by rotary evaporation at reduced pressure and the residue was diluted with saturated sodium bicarbonate aqueous solution (75 mL) and extracted with EtOAc (4 x 50 mL). The extracts were combined, washed with saturated sodium bicarbonate soution (75 mL), dried ($MgSO_4$) and concentrated to afford a tan semi-solid that was recrystallized from EtOAc / hexanes to give **9** as a faint-yellow powder (2.85 g, 58 % yield). Chromatography (acetone / hexanes (30 / 70)) of a portion of this material yielded an analytical sample of **9** as a white powder, mp 169.5−171°C; $[\alpha]_D^{23}$ −4.1° (c = 1.0, $CHCl_3$); FTIR 3241, 1763, 1677 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.50 - 7.35 (5H, m), 6.68 (1H, br s), 4.01 (1H, dd, J = 5.0, 18 Hz), 3.67 (1H, d, J = 18 Hz), 1.85 (3H, s); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 169.7, 166.3, 138.3, 129.1, 128.8, 124.0, 85.0, 44.2, 27.0; EIMS *m/z* calcd. for $C_{11}H_{11}NO_3$ 205.0738, found 205.0739.

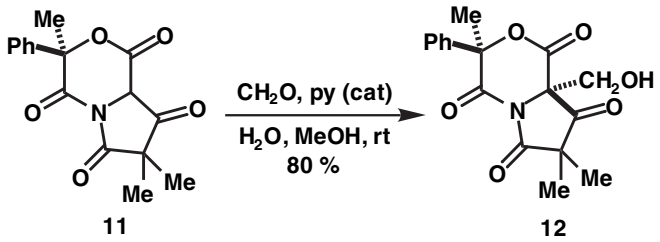


Ethyl (*2'R*)-2,2-dimethyl-3-(2'-methyl-3',6'-dioxo-2'-phenylmorpholin-4'-yl)-3-oxopropionic acid (10). A solution of dimethylmalonic acid monoethyl ester (1.00 g, 6.94 mmol) in benzene (5 mL) containing DMF (35 μL, 0.45 mmol, 6 mol %) was treated with oxalyl chloride (0.93 mL, 10 mmol) dropwise over 5 min. The mixture was stirred at rt for 1.5 h, concentrated, and the residue azeotroped with benzene. The crude acid chloride was taken up in CH_2Cl_2 (6 mL) and added dropwise to a methylene chloride (11 mL) solution of the morpholinedione **9** (1.15 g, 5.60 mmol), 4-(dimethylamino)pyridine (80 mg, 0.65 mmol, 12 mol %) and triethylamine (1.60 mL, 11.5 mmol) at 0 °C. The resulting slurry was stirred at this temperature for an additional 10 min and then at rt for 4 h. The mixture was washed with 0.1 M HCl (2 x 60 mL) and the combined washings were extracted with methylene chloride (4 x 40 mL). The organics were combined, washed with saturated $NaHCO_3$ aqueous solution (2 x 60 mL), dried ($MgSO_4$), concentrated to a 50 mL volume and filtered through a pad of silica gel (1.76 g). The silica gel was washed well with methylene chloride and the filtrate was concentrated to give **10** as a pale yellow syrup (1.89 g, 97 % yield); $[\alpha]_D^{23}$ +81° (c = 1.2, $CHCl_3$); FTIR 1776, 1753, 1723, 1711 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.43 - 7.38 (3H, m), 7.34 - 7.31 (2H, m), 5.04 (1H, d, J = 18 Hz), 4.26 (1H, m), 4.16 (1H, m), 3.51 (1H, d, J = 18 Hz), 1.85 (3H, s), 1.528 (3H, s), 1.525 (3H, s), 1.28 (3H, t, J = 7.1 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 172.8, 172.0, 167.4, 165.5, 137.5, 129.5, 129.2, 123.6, 85.4, 61.3, 53.5, 45.0, 27.5, 23.9, 22.8, 14.1; ESMS *m/z* calcd. for MH^+ $C_{18}H_{22}NO_6$ 348.1446, found 348.1447.



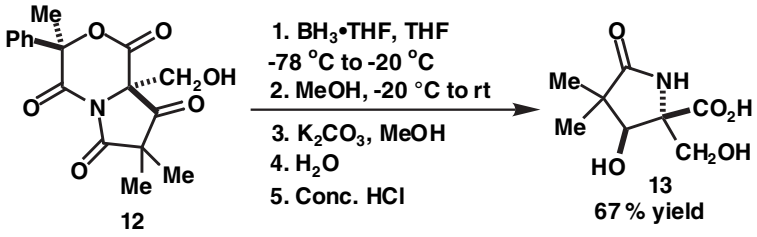
(*3R*)-3,7,7-trimethyl-3-phenylpyrrolo[2,1-c][1,4]oxazine-1,4,6,8-tetraone (11). A methylene chloride solution (110 mL) of the *N*-acylmorpholinedione **10** (2.80 g, 8.06 mmol, dried azeotropically with benzene) was cooled to -78 °C and treated with a freshly prepared methylene chloride solution of $TiCl_4$ (17 mL, 1.0 M) and chlorotrimethylsilane (60 μL, 0.47 mmol, 6 mol %). Triethylamine (1.60 mL, 11.5 mmol) was added dropwise and the resulting purple solution was stirred at -78 °C for 45 min and then at - 41 °C for 4 h 15 min. The final orange-brown mixture was washed with 0.1 M HCl (2 x 100 mL) and the combined wash solutions were extracted with CH_2Cl_2 (3 x 70 mL). The organic layers were collected, dried ($MgSO_4$) and concentrated leaving a red-orange residue that was taken up in ether (120 mL) and extracted with half-saturated sodium bicarbonate solution (2 x 120 mL). The bicarbonate extracts were combined, washed with ether (2 x 100 mL) to remove any remaining neutral or basic organic impurities, adjusted to pH 2 - 3 with concentrated HCl (12 M) and extracted with CH_2Cl_2 (4 x 80 mL). The methylene chloride extracts were combined, dried ($MgSO_4$), concentrated and triturated with hexanes to provide the desired bicyclic tetraone **11** as a faint-yellow foam (2.11 g, 87 %). Treatment of a portion (47 mg, 0.16 mmol) of this mixture with CH_2N_2 (0.2 M in ether, 1 mL, 0.2 mmol) in ether followed by chromatography on silica gel (EtOAc / hexanes (30 / 70)) provided **(*3R*)-8-Methoxy-3,7,7-trimethyl-3-phenyl-7*H*-pyrrolo[2,1-c][1,4]oxazine-1,4,6-trione (A)** as a colorless syrup (33 mg, 67 %); $[\alpha]_D^{23}$ +82° (c = 1.2, $CHCl_3$); FTIR 1795, 1736, 1713 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.42 - 7.35 (5H, m), 4.09 (3H, s), 1.87 (3H, s), 1.31

(3H, s), 1.12 (3H, s); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 178.2, 163.5, 163.1, 156.5, 139.0, 129.1, 129.0, 124.1, 102.8, 85.0, 64.1, 48.1, 26.3, 21.9, 21.2; ESMS *m/z* calcd. for MH^+ $C_{17}H_{18}NO_5$ 316.1184, found 316.1185.



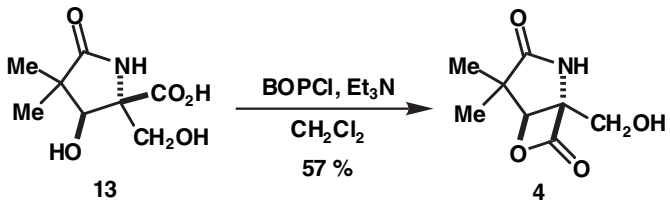
11 12

(*3R*,*8aR*)-8a-Hydroxymethyl-3,7,7-trimethyl-3-phenylpyrrolo[2,1-c][1,4]oxazine-1,4,6,8-tetraone (12). A THF (4.4 mL) solution of **11** (1.31 g, 4.35 mmol) containing pyridine (35 μL, 0.43 mmol, 10 mol %) was treated with a 12 M aqueous solution of formaldehyde (3.6 mL, 10 equiv) with stirring at rt for 40 min. The resulting white slurry was diluted with water (35 mL) and filtered (Celite). The white solid resting on the Celite pad was washed well with water (5 x 30 mL) and the washings were discarded. The solid was dissolved in acetonitrile and the solution diluted with an equal volume of benzene and concentrated. The solid was again redissolved in acetonitrile / benzene (1:1) and the azeotropic drying process was repeated to provide **12** as a colorless powder (1.15 g, 80 % yield); mp 188 - 190 °C; $[\alpha]_D^{23}$ +210° (c = 0.56, CH_3CN); FTIR 3449, 1804, 1776, 1713 cm^{-1}; ^{1}H NMR (CD_3CN, 400 MHz) δ 7.58 – 7.41 (5H, m), 3.85 (1H, br s), 3.57 (1H, d, J = 11 Hz), 2.69 (1H, d, J = 11 Hz), 1.93 (3H, s), 1.35 (3H, s), 1.26 (3H, s); nOe data δ 3.57 (2.69, 20 %; 1.93, 1 %), 2.69 (3.57, 23 %; 1.93, 3 %), 1.93 (3.57, 4 %; 2.69, 2 %); ^{13}C NMR (CD_3CN, 100 MHz) δ 202.7, 174.4, 165.1, 161.1, 140.1, 130.4, 130.0, 124.5, 87.7, 73.8, 65.9, 48.1, 29.4, 20.4; FABMS *m/z* calcd. for $M+Na^+$ 354.0953, found 354.0954.



(*2R,3S*)-3-Hydroxy-2-hydroxymethyl-4,4-dimethyl-5-oxo-2-pyrrolidine-carboxylic acid (13). A 0.6 M THF solution of BH_3·THF complex (4.0 mL, 2.4 mmol, 1.2 equiv) was added slowly along the flask wall to a well stirred solution of **12** (653 mg, 1.97 mmol) in THF (23 mL) at -78 °C. The reaction mixture was stirred at -78 °C for 2 h, warmed slowly to -20 °C over 2 h held at this temperature for 1 h. The reaction was quenched at -20 °C by the addition of methanol (5 mL, dried with 3A molecular sieves) and warmed to rt. The mixture was concentrated and the residue was dissolved in methanol (20 mL) containing anhydrous potassium carbonate (2.80 g, 20.3 mmol, 10 equiv) with stirring at rt for 8 h. Water (1.0 mL) was then introduced with continued stirring at rt for an additional 17 h. The mixture was diluted twofold with methanol, cooled in ice, acidified to pH 2 - 3 with 12 M HCl and concentrated. The solid residue was twice dried azeotropically with a mixture of acetonitrile and benzene (1:1), suspended in chloroform (100 mL) for 12 h, filtered (Celite) and triturated well with chloroform (4 x 20 mL) to remove the atrolactic acid. The Celite and remaining solid were then washed with a solution composed of 10 % triethylamine in CH_2Cl_2 and the filtrate was diluted with an equal volume of benzene and concentrated to afford the triethylammonium salt of the dihydroxy acid **13** as a colorless resin (402 mg, 67 % yield). Regeneration of a sample of the free acid **13** for characterization purposes was accomplished by azeotropic distillation with pyridine (x 2) and then acetic acid (x 2) followed by trituration with THF to afford a white solid, mp 250 °C (dec); $[\alpha]_D^{23}$ +8.4° (c = 1.3, CH_3OH); FTIR 3333, 1681 cm^{-1}; ^1H NMR (CD_3OD, 500 MHz) δ 4.03 (3H, m), 3.68 (1H, d, J = 11 Hz), 1.14 (3H, s), 1.10 (3H, s); ^{13}C NMR (CD_3OD, 100 MHz) δ 183.1,

175.7, 78.0, 69.7, 65.9, 45.7, 24.6, 19.4; ESMS (negative ion) calcd. for M^- $C_8H_{13}NO_5$ 202.0793, found 203.0795.



(*1R, 2S*)-1-Hydroxymethyl-4,4-dimethyl-6-oxa-2-azabicyclo[3.2.0]heptane-3,7-dione (4). A solution of the triethylammonium salt of **13** (360 mg, 1.18 mmol, dried azeotropically with a mixture (1 : 1) of methylene chloride and benzene) and triethylamine (0.33 mL, 2.4 mmol) in methylene chloride (17 mL) was treated with *bis*-(2-oxo-3-oxazolidinonyl)phosphinic chloride (460 mg, 1.80 mmol) with stirring at rt for 90 min. The reaction was quenched by the addition of saturated brine (6 mL) and the layers were separated. The brine solution was extracted with EtOAc (5 x 10 mL) and the combined organics were dried ($MgSO_4$). Concentration in vacuo provided a gummy yellow solid that was purified by chromatography on silica gel (EtOAc) to afford the β-lactone **4** as a white solid (125 mg, 57 % yield); mp 137 - 139 °C; $[\alpha]_D^{23}$ +100° (c = 0.52, CH_3CN); FTIR (CCl4) 3202, 3085, 1840, 1699 cm^{-1}; ^1H NMR (CD_3CN, 600 MHz) δ 6.86 (1H, br s), 4.77 (1H, s), 3.93 (1H, dd, J = 6.6, 12 Hz), 3.73 (1H, dd, J = 5.1, 12 Hz), 3.55 (1H, t, J = 5.7 Hz), 1.17 (3H, s), 1.14 (3H, s); ^{13}C NMR (CD_3CN, 100 MHz) δ 180.3, 170.0, 81.4, 76.6, 58.5, 43.2, 24.1, 17.0; CIMS *m/z* calcd. for $M+NH_4^+$ $C_8H_{13}N_2O_5$ 203.1032, found 203.1032.