

Stereoselective Synthesis of Optically Active β -Lactams, Potential Inhibitors of Pilus Assembly in Pathogenic Bacteria.

Hans Emtenäs^a, Gabe Soto^b, Scott J. Hultgren^b, Garland R. Marshall^c and Fredrik Almqvist^{a*}

^aOrganic Chemistry, Umeå University, S-901 87 Umeå, Sweden. ^bDepartment of Molecular Microbiology, and ^cCenter of Molecular Design, Department of Molecular Biology and Pharmacology, Washington University, 660 S. Euclid, Saint Louis, Missouri, 63130, USA

fredrik.almqvist@chem.umu.se

Supporting Information

General. All reactions were carried out under an inert atmosphere with dry solvents under anhydrous conditions, unless otherwise stated. CH₂Cl₂ were distilled from calcium hydride and THF from potassium, respectively. Benzene and Toluene was distilled from sodium. TLC was performed on Silica Gel 60 F₂₅₄ (Merck) with detection by UV light and staining with a solution of anisaldehyde (26 ml), glacial acetic acid (11 ml) and concentrated sulphuric acid (35 ml) in 95 % ethanol (960 ml). Flash column chromatography (eluents given in brackets) was performed on silica gel (Matrex, 60 Å, 35-70 µm, Grace Amicon). Centrifugal preparative TLC was performed using rotors coated with silica gel 60 PF₂₅₄ containing CaSO₄ (Merck). The moving bands were visualized using UV light.

The ¹H and ¹³C NMR spectra were recorded on a Bruker DRX-400 for solutions in CDCl₃ [residual CHCl₃ (δ_H 7.26 ppm) or CDCl₃ (δ_C 77.0 ppm) as internal standard at 298 K. First-order chemical shifts and coupling constants were obtained from one-dimensional spectra. Proton and carbon resonances were assigned from appropriate combinations of COSY and HMBC.

IR spectra were recorded on an ATI Mattson Genesis Series FTIRTM spectrometer. Optical rotations were measured with a Perkin-Elmer 343 polarimeter. Positive fast atom bombardment mass spectra (FABMS) were recorded on a JEOL SX102 A mass spectrometer. Ions for FABMS were produced by a beam of Xenon atoms (6 keV) from a matrix of glycerol and thioglycerol.

(2R,5S,6R)-6-Benzoyl-7-oxo-4-thia-1-aza-bicyclo[3.2.0]heptane-2-carboxylic acid methyl ester (3a).

Meldrum's acid derivative **1a** (1027 mg, 4.14 mmol) and thiazoline **2** (394 mg, 2.72 mmol) were dissolved in dry benzene (44 ml) and cooled to 5 °C. HCl gas was bubbled through the mixture for 10 minutes. The resulting turbid mixture was heated for 1.5 hours at 79 °C and then cooled to room temperature. The resulting mixture was diluted with ethyl acetate and washed with ice cooled water and brine. The water phase was extracted twice with CH₂Cl₂ and the combined organic extracts were dried with Na₂SO₄, filtered and concentrated. Flash chromatography (heptane:ethyl acetate 1:1 → 3:7) gave **3a** (643 mg, 81 %): $[\alpha]_D^{20}$ 38° (c 2.60, CHCl₃); IR λ 3062, 3004, 2952, 1739, 1658, 1604, 1387 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 7.70 (m, 2H), 7.40-7.51 (m, 3H), 6.68 (s, 1H), 6.00 (s, 1H), 5.35 (d, 1H, J = 6.40 Hz), 3.81 (s, 1H), 3.55 (dd, 1H, J_1 = 11.25 Hz, J_2 = 6.40 Hz), 3.30 (d, 1H, J = 11.25 Hz); ¹³C NMR (100 MHz, CDCl₃) δ 168.7, 164.3, 161.5, 131.7, 130.8, 128.8, 126.6, 97.7, 94.4, 60.9, 53.1, 32.1.

HRMS (EI+) Calcd. for C₁₄H₁₃NO₄S: 291.0565 Observed 291.0567

(2R,5S,6R)-6-(Naphthalene-1-carbonyl)-7-oxo-4-thia-1-aza-bicyclo[3.2.0]heptane-2-carboxylic acid methyl ester (3b). Meldrum's acid derivative **1b** (630 mg, 2.11 mmol) and thiazoline **2** (185 mg, 1.27 mmol) were dissolved in dry benzene (28 ml) and cooled to 5 °C. HCl gas was bubbled through the mixture for 10 minutes. The resulting turbid mixture was heated for 1.5 hours at 79 °C and then cooled to room temperature. The resulting mixture was diluted with ethyl acetate and washed with ice cooled water and brine. The water phase was extracted twice with CH₂Cl₂ and the combined organic extracts were dried with Na₂SO₄, filtered and concentrated. Flash chromatography

(heptane:ethyl acetate 1:1 -> 3:7) gave **3b** (403 mg, 93 %) as a white foam: $[\alpha]_D^{20}$ 2.7° (c 1.77, CHCl₃); IR λ 1739, 1664, 1508, 1388, 1211, 977, 773 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 8.14 (d, 1H, J = 8.23 Hz), 7.96 (d, 1H, J = 8.23 Hz), 7.89 (m, 1H), 7.66 (dd, 1H, J_1 = 7.23 Hz, J_2 = 1.19 Hz), 7.46-7.59 (m, 3H), 6.93 (s, 1H), 5.89 (s, 1H), 5.43 (d, 1H, J = 6.31 Hz), 3.84 (s, 3H), 3.60 (dd, 1H, J_1 = 11.25 Hz, J_2 = 6.40 Hz), 3.32 (d, 1H, J = 11.25); ¹³C NMR (100 MHz, CDCl₃) δ 169.7, 165.7, 161.2, 133.7, 131.7, 130.6, 129.7, 128.2, 127.3, 126.4, 124.9, 124.8, 103.4, 94.4, 60.9, 53.2, 32.1
HRMS (FAB+) Calcd. for C₁₈H₁₆NO₄S: 342.0800 Observed 342.0803.

(2R,5S,6R)-6-(Naphthalen-1-yl-acetyl)-7-oxo-4-thia-1-aza-bicyclo[3.2.0]heptane-2-carboxylic acid methyl ester (3c). Meldrum's acid derivative **1c** (1250 mg, 4.00 mmol) and thiazoline **2** (440 mg, 3.03 mmol) were dissolved in dry benzene (55 ml) and cooled to 5 °C. HCl gas was bubbled through the mixture for 15 minutes. The resulting turbid mixture was heated for 3 hours at 79 °C and then cooled to room temperature. The resulting mixture was diluted with ethyl acetate and washed with ice cooled water and brine. The water phase was extracted twice with CH₂Cl₂ and the combined organic extracts were dried with Na₂SO₄, filtered and concentrated. Flash chromatography (heptane:ethyl acetate 1:1 -> 3:7) gave **3c** (660 mg, 62 %) as a white foam: $[\alpha]_D^{20}$ -3.75° (c 0.40, CHCl₃); IR λ 3008, 2952, 1743, 1664, 1396, 1215, 968, 752 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 7.88 (m, 2H), 7.81, (d, 1H, J = 8.14 Hz), 7.51(m, 2H), 7.43 (m, 1H), 7.36 (d, 1H, J = 6.77 Hz), 6.55 (s, 1H), 5.27 (d, 1H, J = 6.31 Hz), 5.10 (s, 1H), 4.00 (s, 2H), 3.75 (s, 3H), 3.49 (dd, 1H, J_1 = 11.16 Hz, J_2 = 6.40 Hz), 3.23 (d, 1H, J = 11.16 Hz); ¹³C NMR (100 MHz, CDCl₃) δ 169.5, 168.9, 160.7, 133.9, 131.8, 130.5, 128.8, 128.4, 128.1, 126.5, 125.9, 125.4, 123.4, 101.1, 94.0, 60.7, 53.0, 36.7, 31.9.
HRMS (FAB+) Calcd. for C₁₉H₁₈NO₄S 356.0957 Observed 356.0960.

(2R,5S,6R)-6-(Naphthalen-2-yl-acetyl)-7-oxo-4-thia-1-aza-bicyclo[3.2.0]heptane-2-carboxylic acid methyl ester (3d). Meldrum's acid derivative **1d** (1263 mg, 4.04 mmol) and thiazoline **2** (392 mg, 2.70 mmol) were dissolved in dry benzene (60 ml) and cooled to 5 °C. HCl gas was bubbled through the mixture for 10 minutes. The resulting turbid

mixture was heated for 2.5 hours at 79 °C and then cooled to room temperature. The resulting mixture was diluted with ethyl acetate and washed with ice cooled water and brine. The water phase was extracted twice with CH₂Cl₂ and the combined organic extracts were dried with Na₂SO₄, filtered and concentrated. Flash chromatography (heptane:ethyl acetate 1:1 -> 3:7) gave **3d** (622 mg, 65 %): $[\alpha]_D^{20}$ 3.3° (c 2.96, CHCl₃); IR λ 3049, 3010, 1741, 1666, 1392, 1209, 964, 813, 746 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 7.80 (m, 3H), 7.66 (s, 1H), 7.48 (m, 2H), 7.32 (dd, 1H, J_1 = 8.51 Hz, J_2 = 1.37 Hz), 6.50 (s, 1H), 5.31 (s, 1H), 5.28 (d, 1H, J = 6.31 Hz), 3.74 (s, 3H), 3.69 (s, 2H), 3.44 (dd, 1H, J_1 = 11.16 Hz, J_2 = 6.50 Hz), 3.20 (d, 1H, J = 11.16 Hz); ¹³C NMR (100 MHz, CDCl₃) δ 169.5, 168.9, 160.7, 133.3, 132.4, 130.1, 128.4, 127.9, 127.6, 127.5, 126.9, 126.2, 125.9, 100.9, 94.0, 60.6, 52.9, 39.6, 31.7. HRMS (CI+) Calcd. for C₁₉H₁₈NO₄S: 356.0957 Observed 356.0956.

(2R,5S,6R)-6-Cyclohexanecarbonyl-7-oxo-4-thia-1-aza-bicyclo[3.2.0]heptane-2-carboxylic acid methyl ester (3e). Meldrum's acid derivative **1e** (1210 mg, 4.76 mmol) and thiazoline **2** (435 mg, 3.00 mmol) were dissolved in dry benzene (50 ml) and cooled to 5 °C. HCl gas was bubbled through the mixture for 12 minutes. The resulting turbid mixture was heated for 2 hours at 79 °C and then cooled to room temperature. The resulting mixture was diluted with ethyl acetate and washed with ice cooled water and brine. The water phase was extracted twice with CH₂Cl₂ and the combined organic extracts were dried with Na₂SO₄, filtered and concentrated. Flash chromatography (heptane:ethyl acetate 1:1 -> 3:7) of the residue and then centrifugal preparative TLC 2mm (heptane:ethyl acetate 1:1) gave **3e** (644 mg, 72 %): $[\alpha]_D^{20}$ 7.4° (c 2.28, CHCl₃); IR λ 2929, 2852, 1736, 1657, 1610, 1404, 1296, 1223, 1005, 960, 933, 804, 756 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 6.45 (s, 1H), 5.30 (s, 1H), 5.28 (d, 1H, J = 6.31 Hz), 3.77 (s, 3H), 3.48 (dd, 1H, J_1 = 11.25 Hz, J_2 = 6.50 Hz), 3.23 (d, 1H, J = 11.25), 2.14 (m, 1H), 1.82 (m, 4H), 1.69 (m, 1H), 1.11-1.36 (m, 5H); ¹³C NMR (100 MHz, CDCl₃) δ 174.4, 169.8, 161.3, 97.9, 93.8, 60.7, 53.0, 41.7, 31.9, 30.1, 29.8, 35.8, 25.7, 25.6. HRMS (EI+) Calcd. for C₁₄H₁₉NO₄S: 297.1035 Observed 297.1035.

(2R,5S,6R)-6-Hexanoyl-7-oxo-4-thia-1-aza-bicyclo[3.2.0]heptane-2-carboxylic acid methyl ester (3f).

Meldrum's acid derivative **1f** (1133 mg, 4.67 mmol) and thiazoline **2** (443 mg, 3.05 mmol) were dissolved in dry benzene (50 ml) and cooled to 5 °C. HCl gas was bubbled through the mixture for 12 minutes. The resulting turbid mixture was heated for 2 hours at 79 °C and then cooled to room temperature. The resulting mixture was diluted with ethyl acetate and washed with ice cooled water and brine. The water phase was extracted twice with CH₂Cl₂ and the combined organic extracts were dried with Na₂SO₄, filtered and concentrated. Flash chromatography (heptane:ethyl acetate 1:1 -> 3:7) of the residue and then centrifugal preparative TLC 2mm (heptane:ethyl acetate 1:1) gave **3f** (696 mg, 80 %): $[\alpha]_D^{20}$ 7.3° (c 1.94, CHCl₃); IR λ 2954, 2931, 1743, 1670, 1614, 1394, 1296, 1211, 968, 810 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 6.48 (s, 1H), 5.31 (s, 1H), 5.28 (d, 1H, *J* = 6.40 Hz), 3.77 (s, 3H), 3.47 (dd, 1H, *J*₁ = 11.25 Hz, *J*₂ = 6.40 Hz), 3.22 (d, 1H, *J* = 11.25 Hz), 2.23 (m, 2H), 1.53 (p, 2H, *J* = 7.50 Hz), 1.30 (m, 4H), 0.88 (t, 3H, *J* = 7.20 Hz); ¹³C NMR (100 MHz, CDCl₃) δ 170.6, 169.7, 161.0, 99.7, 93.7, 60.7, 53.0, 33.1, 31.9, 31.1, 25.8, 22.2, 13.9.

HRMS (EI+) Calcd. for C₁₃H₁₉NO₄S: 285.1035 Observed 285.1033

(2R,5S,6R)-6-Acetyl-7-oxo-4-thia-1-aza-bicyclo[3.2.0]heptane-2-carboxylic acid methyl ester (3g).

Meldrum's acid derivative **1g** (738 mg, 3.96 mmol) and thiazoline **2** (377 mg, 2.60 mmol) were dissolved in dry benzene (41 ml) and cooled to 5 °C. HCl gas was bubbled through the mixture for 10 minutes. The resulting turbid mixture was heated for 2 hours at 79 °C and then cooled to room temperature. The resulting mixture was diluted with ethyl acetate and washed with ice cooled water and brine. The water phase was extracted twice with CH₂Cl₂ and the combined organic extracts were dried with Na₂SO₄, filtered and concentrated. Flash chromatography (heptane:ethyl acetate 1:1 -> 3:7) gave **3g** (227 mg, 38 %): $[\alpha]_D^{20}$ 12° (c 2.76, CHCl₃); IR λ 3008, 2953, 1739, 1666, 1396, 1356, 1209, 1157, 966, 812, 748 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 6.49 (s, 1H), 5.30 (s, 1H), 5.26 (d, 1H, *J* = 6.31 Hz), 3.75 (s, 3H), 3.45 (dd, 1H, *J*₁ = 11.34 Hz, *J*₂ = 6.31 Hz), 3.21 (d, 1H,

$J = 11.34$ Hz), 1.98 (s, 3H): ^{13}C NMR (100 MHz, CDCl_3) δ 169.6, 166.9, 160.8, 100.4, 93.6, 60.7, 53.0, 31.8, 19.3.

HRMS (EI+) Calcd. for $\text{C}_9\text{H}_{11}\text{NO}_4\text{S}$: 229.0397 Observed 229.0407.

(2R,5S,6R)-6-Benzoyl-7-oxo-4-thia-1-aza-bicyclo[3.2.0]heptane-2-carbaldehyde (5a).

To a solution of **3a** (377 mg, 1.29 mmol) in THF (25 ml) was added DIBAL-H (1330 μl , 1.5 M in toluen) at -78°C . After stirring for 2h a further amount of DIBAL-H (1000 μl , 1.5 M in toluen) was added at -78°C . 30 min later the mixture was quenched with sat. NH_2Cl (aq) (2000 μl) and the mixture was allowed to reach RT. Precipitated material was filtered off and the filtrate was washed with water and brine. The aqueous layer was extracted with several portions of CH_2Cl_2 and the combined organic layers were dried with Na_2SO_4 , filtered and concentrated. Flash Chromatography (heptane:ethyl acetate 1:2) gave **5a** as an oil (165 mg, 49 %): $[\alpha]_{\text{D}}^{20}$ 12° (c 1.36, CHCl_3); IR λ 1734, 1651, 1448, 1383, 1011, 928, 768 cm^{-1} : ^1H NMR (400 MHz, CDCl_3) δ 9.66 (s, 1H), 7.70 (m, 2H), 7.37-7.53 (m, 3H), 6.65 (s, 1H), 6.03 (s, 1H), 5.18 (d, 1H, $J = 6.40$ Hz), 3.35-3.43 (m, 2H): ^{13}C NMR (100 MHz, CDCl_3) δ 197.1, 164.9, 162.3, 1318, 130.6, 128.7, 126.6, 97.6, 94.4, 67.4, 28.4.

HRMS (EI+) Calcd. for $\text{C}_{13}\text{H}_{11}\text{NO}_3\text{S}$: 261.0460 Observed 261.0461.

(2R,5S,6R)-6-(Naphthalene-1-carbonyl)-7-oxo-4-thia-1-aza-bicyclo[3.2.0]heptane-2-carbaldehyde (5b). To a solution of **3b** (155 mg, 0.454 mmol) in THF (10 ml) was added DIBAL-H (550 μl , 1.5 M in toluen) at -78°C . After stirring for 2h a further amount of DIBAL-H (550 μl , 1.5 M in toluen) was added at -78°C . 30 min later the mixture was quenched with sat. Rochell's salt (aq) (6ml) and the mixture was allowed to reach RT. Precipitated material was filtered off and the filtrate was washed with water and brine. The aqueous layer was extracted with several portions of CH_2Cl_2 and the combined organic layers were dried with Na_2SO_4 , filtered and concentrated. Flash chromatography (heptane:ethyl acetate 1:2) gave **5b** (44 mg, 31 %): $[\alpha]_{\text{D}}^{20}$ 0.37° (c 1.36, CHCl_3); IR λ 1733, 1643, 1384, 979, 773 cm^{-1} : ^1H NMR (400 MHz, CDCl_3) δ 9.72 (s, 1H), 8.14 (m, 1H), 7.97 (m, 1H), 7.90 (m, 1H), 7.68 (m, 1H), 7.47-7.61 (m, 3H), 6.91 (s,

1H), 5.94 (s, 1H), 5.28 (dd, 1H, $J_1 = 6.68$ Hz, $J_2 = 0.91$ Hz), 3.46-3.53 (m, 1H), 3.40-3.44 (m, 1H): ^{13}C NMR (100 MHz, CDCl_3) δ 197.00, 166.40, 161.92, 133.67, 131.88, 130.51, 129.45, 128.70, 128.28, 127.36, 126.45, 124.87, 124.75, 103.29, 94.47, 67.41, 28.39.

HRMS (FAB+) Calcd. for $\text{C}_{17}\text{H}_{14}\text{NO}_3\text{S}$: 312.0694 Observed 312.0691.

(2R,5S,6R)-6-(Naphthalen-1-yl-acetyl)-7-oxo-4-thia-1-aza-bicyclo[3.2.0]heptane-2-carbaldehyde (5c).

To a solution of **3c** (127 mg, 0.357 mmol) in THF (6.8 ml) was added DIBAL-H (360 μl , 1.5 M in toluen) at -78°C . After stirring for 2h a further amount of DIBAL-H (250 μl , 1.5 M in toluen) was added at -78°C . 30 min later the mixture was quenched with sat. NH_4Cl (aq) (700 μl) and the mixture was allowed to reach RT. Precipitated material was filtered off and the filtrate was washed with water and brine. The aqueous layer was extracted with several portions of CH_2Cl_2 and the combined organic layers were dried with Na_2SO_4 , filtered and concentrated. Centrifugal preparative TLC 1mm (heptane:ethyl acetate 1:1 \rightarrow 1:2) gave **5c** (51.7 mg, 44 %): $[\alpha]_{\text{D}}^{20} 0^\circ$ (c 1.40, CHCl_3); IR λ 3461, 3215, 2908, 1736, 1643, 1510, 1392, 1090, 1066, 964, 777 cm^{-1} : ^1H NMR (400 MHz, CDCl_3) δ 9.57 (s, 1H), 7.85-7.91 (m, 2H), 7.82 (m, 1H), 7.48-7.57 (m, 2H), 7.41-7.47 (m, 1H), 7.35-7.40 (m, 1H), 6.52 (s, 1H), 5.15 (s, 1H), 5.11 (d, 1H, $J = 6.50$ Hz), 4.02 (d, 2H, 6.77), 3.29-3.45 (m, 2H): ^{13}C NMR (100 MHz, CDCl_3) δ 197.6, 169.9, 161.6, 134.5, 131.9, 130.5, 129.0, 128.6, 128.3, 126.7, 126.1, 125.6, 123.5.

HRMS (FAB+) Calcd. for $\text{C}_{18}\text{H}_{16}\text{NO}_3\text{S}$: 326.08509 Observed 326.0848.

(2R,5S,6R)-6-(Naphthalen-2-yl-acetyl)-7-oxo-4-thia-1-aza-bicyclo[3.2.0]heptane-2-carbaldehyde (5d). To a solution of **3d** (260 mg, 0.732 mmol) in CH_2Cl_2 (18 ml) was added DIBAL-H (1150 μl , 1.5 M in toluen) at -78°C . After stirring for 2h a further amount of DIBAL-H (300 μl , 1.5 M in toluen) was added at -78°C . 30 min later the mixture was quenched with sat. Rochelle Salt (aq) (7.5ml) and sat NH_4Cl (aq) (10ml), then the mixture was allowed to reach RT. Precipitated material was filtered off and the filtrate was wahed with water and brine. The aqueous layer was extracted with several

portions of CH_2Cl_2 and the combined organic layers were dried with Na_2SO_4 , filtered and concentrated. Flash chromatography (heptane:ethyl acetate 3:7) gave **5d** (97 mg, 41 %): $[\alpha]_{\text{D}}^{20}$ 0.7° (c 0.85, CHCl_3); IR λ 3050, 1733, 1658, 1390, 1081, 962, 811 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3) δ 9.59 (s, 1H), 7.82 (m, 3H), 7.68 (m, 1H), 7.49 (m, 2H), 7.33 (m, 1H), 6.48 (s, 1H), 5.37 (s, 1H), 5.12 (dd, 1H, $J_1 = 6.31$ Hz, $J_2 = 1.37$ Hz), 3.73 (s, 2H), 3.29-3.36 (m, 2H); ^{13}C NMR (100 MHz, CDCl_3) δ 197.01, 169.82, 161.57, 133.48, 132.60, 132.02, 128.60, 128.09, 127.70, 127.62, 127.02, 126.40, 126.06, 101.07, 94.22, 67.28, 39.85, 28.17.

HRMS (FAB+) Calcd. for $\text{C}_{18}\text{H}_{16}\text{NO}_3\text{S}$: 326.0851 Observed 326.0866.