Synthesis of a Directly Connected Thiazole−Oxazole Ring System Present in Microcin B17

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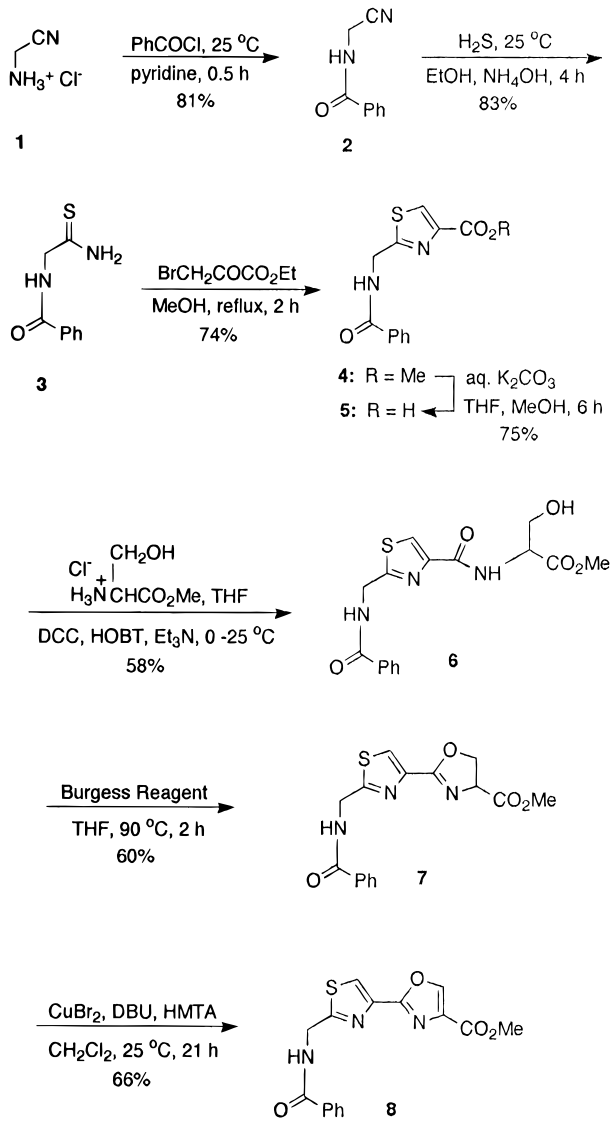
Microcin B17 (McB17) is a 43 residue peptide antibiotic ribosomally synthesized in several strains of *Escherichia coli*.[1] Recently, we[2] and others[3] have reported that fourteen of these residues are posttranslationally modified; two Gly-Cys and Gly-Ser dipeptides lead, respectively, to two isolated thiazole and oxazole rings, whereas two tripeptides Gly-Cys-Ser and Gly-Ser-Cys produce two directly connected thiazole−oxazole ring systems. The identification of thiazoles and oxazoles as the modified forms clearly indicates that McB17 is structurally unrelated to the two known classes of DNA gyrase inhibitors, namely quinolones and coumarins. Yet McB17 is a strong DNA gyrase inhibitor leading to the inhibition of DNA replication and, hence, cell death.[4] While the exact roles of the thiazole and oxazole rings are not yet understood, genetic and biochemical studies indicate that they may be structurally and functionally important.[2] Thus, we undertook their synthesis.

Although a variety of methods were available for the preparation of isolated thiazole[5] and oxazole[6] rings, to the best of our knowledge, the synthesis of a directly connected thiazole−oxazole ring system has never been reported in the literature.[7−10] Here we report the synthesis of **8** (Scheme 1), an appropriately protected and directly connected thiazole−oxazole ring system present in McB17. Coincidentally, **4** represents a protected version of the aforementioned Gly-Cys modified dipeptide found in McB17.

Commercially available aminoacetonitrile hydrochloride (**1**) served as our starting material. Although both

Scheme 1



N-acetyl and *N*-benzoyl derivatives could be used in the synthesis described in Scheme 1, the latter were easier to crystallize and purify throughout the series. Therefore, **1** was converted to **2** under standard benzoylation conditions (benzoyl chloride, pyridine, 25 °C, 0.5 h, 81%).[12] Hydrogen sulfide[13] treatment of an aqueous ethanolic ammonia solution of **2** provided the desired thionamide **3**, which on condensation with ethyl bromopyruvate[14] in boiling methanol afforded the fully protected thiazole amino acid **4**. It should be noted that **4** was indeed a methyl ester due to complete transesterification in boiling methanol. Selective hydrolysis of the

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ester group (aqueous K_2CO_3, THF, MeOH, 25 °C, 6 h, 75%) provided **5**, which was then elaborated, as described below and in Scheme 1, to construct the oxazole ring.

Although the coupling of **5** with DL-serine methyl ester proceeded under standard solution phase peptide coupling conditions, we have found that **6** exhibits a much higher than expected tendency to eliminate. Thus, longer reaction times and/or excessive use of triethylamine must be avoided to obtain reasonable yields of the coupling product.[14] Further, this higher tendency for elimination also made the conversion of **6** to **7** the most challenging step in our synthetic sequence. The tosylate, mesylate, or halides desired for intramolecular cyclization of **6** were not useful, as elimination was the exclusive pathway. Similar results were obtained under Mitsunobu conditions for direct cyclization.[15] Fortunately, the Burgess reagent,[16] as recently reported by Wipf and Miller,[17] induced the desired cyclization to **7** in 60% yield. It was important to use a somewhat higher reaction temperature and shorter reaction time than reported[17] to minimize elimination. With dihydrooxazole **7** in hand, we then examined conditions for its oxidative aromatization.[6b,18,19] The recently reported[19] $CuBr_2$−DBU system worked the best, thereby completing the synthesis of **8** in seven steps with an overall yield of 8.5%.

Experimental Section

General. Unless otherwise noted, all synthetic reactions were performed in oven-dried glassware under a positive atmosphere of dry nitrogen, and their progress was monitored by thin layer chromatography (TLC), using E. Merck silica gel 60F glass plates (0.25 mm thick). Chromatographic purifications were performed on silica gel according to the Still protocol. Solvents were freshly distilled prior to use as follows: THF from sodium benzophenone ketyl, methanol from magnesium methoxide, and dichloromethane, DBU, pyridine, and triethylamine from calcium hydride. All other solvents and reagents were used as purchased from the manufacturers. The term *in vacuo* refers to solvent removal using a rotary evaporator, followed by drying under high vacuum (ca. 0.5 mmHg) for several hours. ^{1}H NMR spectra were obtained at 300 MHz and ^{13}C NMR spectra at 75.2 MHz. Elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, TN.

Preparation of 2-Benzamidoacetonitrile (2). Aminoacetonitrile hydrochloride (**1**) (8.0 g, 87 mmol) was placed in a flask equipped with an addition funnel. Pyridine (50 mL) was carefully added to obtain a solution, and to this was added benzoyl chloride (10.5 mL, 90 mmol) dropwise over 0.5 h. After stirring overnight at room temperature, water (50 mL) was carefully added; pyridinium hydrochloride dissolved while the product precipitated as a white solid. The precipitate was collected by filtration, washed with water, and recrystallized from 95% ethanol to obtain **2** (11.3 g, 81%) as white crystals, mp 140 °C (lit.[12] 144 °C). ^{1}H NMR (DMSO-d_6): δ 9.21 (1H, s), 7.80−7.95 (2H, m), 7.48−7.65 (3H, m), 4.32 (2H, d, J = 3.90 Hz); ^{13}C NMR (DMSO-d_6): δ 166.60, 132.79, 131.91, 128.47, 127.29, 117.61, 27.68.

Preparation of 2-Benzamidothioacetamide (3).[13] Hydrogen sulfide gas was bubbled for 4 h through a solution of **2** (21.0 g, 0.13 mol) in 200 mL of absolute ethanol and 40 mL of NH_4OH. After bubbling argon for 2 h to drive off excess H_2S, the reaction volume was reduced to about 100 mL *in vacuo*. Water (5 mL) was added and the flask slightly heated to dissolve any solid material that had settled. Yellow crystals appeared when the flask was set aside at room temperature overnight, and these were collected by filtration and washed with 95% ethanol to obtain **3** (21.1 g, 83%), mp 149−150 °C. ^{1}H NMR (DMSO-d_6): δ 9.72 (1H, s), 9.17 (1H, s), 8.84 (1H, t, J = 6.45 Hz), 7.45−7.92 (5H, m), 4.16 (2H, d, J = 6.00 Hz); ^{13}C NMR (DMSO-d_6): δ 203.45, 166.36, 133.99, 131.27, 128.12, 127.43, 49.60. Anal. Calcd for $C_9H_{10}N_2OS$: C, 55.65; H, 5.19; N, 14.42. Found: C, 55.80; H, 5.53; N, 14.33.

Preparation of Methyl 2-(Benzamidomethyl)thiazole-4-carboxylate (4).[13] Ethyl bromopyruvate (11.56 g, 59.3 mmol) was added dropwise, over a 0.5 h period, to a 50 °C solution of **3** (11.5 g, 59.3 mmol) in methanol (100 mL) after which the reaction was refluxed for an additional 2 h. Most of the product crystallized when the solution was set aside at room temperature overnight, and this was collected by filtration. The filtrate was evaporated, redissolved in benzene, washed successively with saturated aqueous sodium bicarbonate and water, dried over anhydrous sodium sulfate, and evaporated. Recrystallization of this residue from methanol provided an additional crop. The total yield of **4** was 12.1 g (74%), mp 145−146 °C. ^{1}H NMR ($CDCl_3$): δ 8.16 (1H, s), 7.78−7.84 (2H, m), 7.42−7.60 (3H, m), 7.03 (1H, br), 4.98 (2H, d, J = 5.7 Hz), 3.95 (3H, s); ^{13}C NMR ($CDCl_3$): δ 168.38, 167.55, 161.67, 146.39, 133.37, 132.04, 128.69, 128.56, 127.15, 52.49, 41.36. Mass spec (m/z): 275 (M^+), 257 (10%), 206 (12%), 170 (55%), 155 (10%), 138 (53%), 104 (100%), 76 (82%), 51 (55%). Anal. Calcd for $C_{13}H_{12}N_2O_3S$: C, 56.51; H, 4.38; N, 10.14. Found: C, 56.87; H, 4.65; N, 9.90.

Preparation of 2-(Benzamidomethyl)thiazole-4-carboxylic Acid (5). A solution of **4** (11.5 g, 41.7 mmol) in THF (75 mL), methanol (375 mL), and 1 M aqueous potassium carbonate (150 mL) was stirred at room temperature for 6 h and then acidified by addition of 1 M HCl (450 mL). The resulting mixture was extracted with one 300 mL and then two 200 mL portions of dichloromethane. The combined organic extracts were dried over sodium sulfate and concentrated and the residue recrystallized from 200 mL of hot acetonitrile/water (20/1, v/v) to obtain **5** (8.2 g, 75%) as a white solid, mp 214−215 °C. ^{1}H NMR (DMSO-d_6): δ 9.47 (1H, t, J = 6.03 Hz), 8.36 (1H, s), 7.51−7.93 (5H, m), 4.75 (2H, d, J = 6.00 Hz); ^{13}C NMR (DMSO-d_6): δ 170.06, 166.57, 162.00, 146.65, 133.49, 131.64, 128.73, 128.42, 127.25, 41.03.

Preparation of 2-(Benzamidomethyl)-4-[*N*-[1-(methoxycarbonyl)-2-hydroxyethyl]carbamoyl]thiazole (6). To a 0 °C solution of **5** (2.62 g, 10 mmol) in THF (40 mL) were added, sequentially, 1-hydroxybenzotriazole hydrate (HOBT) (1.77 g, 11.6 mmol), DL-serine methyl ester hydrochloride (1.62 g, 10.5 mmol), and triethylamine (3 mL, 21.7 mmol). The resulting slurry was stirred for 5 min, and then dicyclohexylcarbodiimide (DCC) (2.165 g, 10.5 mmol) was added rapidly. The reaction mixture was then stirred for 4 h at 0 °C and then at room temperature for 10 h. The resulting slurry was cooled to 0 °C, diluted with ethyl acetate (40 mL), stirred for 15 min, and then filtered. The filtrate was concentrated *in vacuo*, and the residue was purified by silica gel flash chromatography (acetone/ethyl acetate/hexane = 1/1/1) and recrystallized from methanol to obtain **6** (2.1 g, 58%). TLC: R_f (silica gel, acetone/ethyl acetate/hexane = 1/1/1) = 0.34. ^{1}H NMR ($CDCl_3$): δ 8.19 (1H, d, J = 6.45 Hz), 7.89−7.92 (2H, m), 7.84 (1H, s), 7.63 (1H, br), 7.40−7.55 (3H, m), 4.85 (3H, m), 4.10 (2H, d, J = 2.4 Hz), 3.82 (3H, s); ^{13}C NMR ($CDCl_3$): δ 170.82, 168.32, 167.61, 161.02, 148.26, 133.25, 132.10, 128.74, 127.27, 125.05, 63.11, 54.85, 52.89, 41.25.

Preparation of 2-(Benzamidomethyl)-4-[2′-[4′-(carbomethoxy)-4′,5′-dihydrooxazolyl]]thiazole (7). A solution of methyl *N*-[(triethylammonio)sulfonyl]carbamate, Burgess reagent,[16] (318 mg, 1.3 mmol) in THF (10 mL) was added dropwise over 20 min to a room temperature solution of **6** (427 mg, 1.2 mmol) in THF (5 mL). The Pyrex tube containing this mixture was then capped, and heated at 90 °C for 2 h. Upon cooling to room temperature, the reaction mixture was concentrated, purified by flash chromatography (acetone/ethyl acetate/hexane = 1/1/1), and recrystallized from methanol to obtain **7** (179 mg, 60%) as a white crystalline solid, mp 193−194 °C. TLC: R_f (silica gel, acetone/ethyl acetate/hexane = 1/1/1) = 0.29. ^{1}H NMR ($CDCl_3$): δ 7.96 (1H, s), 7.75−7.80 (2H, m), 7.38−7.53 (3H, m), 7.00 (1H, br), 4.95 (4H, d, J = 6.00 Hz), 4.72 (1H, t, J = 11.79 Hz), 3.78 (3H, s); ^{13}C NMR ($CDCl_3$): δ 171.21, 167.46, 133.38, 132.08, 132.01, 128.78, 128.69, 127.25, 127.15, 125.03,

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53.12, 52.78, 45.07, 41.35, 41.20. Anal. Calcd for $C_{16}H_{15}N_3O_4S$: C, 55.68; H, 4.46; N, 12.38. Found: C, 55.64; H, 4.38; N, 12.17.

Preparation of 2-(Benzamidomethyl)-4-[2′-[4′-(carbomethoxy)oxazolyl]]thiazole (8).[19] To a suspension of $CuBr_2$ (155 mg, 0.7 mmol) in dry, deoxygenated dichloromethane (15 mL) was added hexamethylenetetramine (HMTA) (97 mg, 0.7 mmol) followed by DBU (106 mg, 0.7 mmol). The resulting warm, dark brown solution was cooled in a water bath for 10 min, and to this was added solid **7** (120 mg, 0.35 mmol). After 21 h at room temperature, the solvent was removed *in vacuo* and the residue partitioned with equal amounts (v/v) of ethyl acetate and saturated aqueous NH_4Cl/concentrated NH_4OH (1:1). The aqueous layer was extracted further with ethyl acetate (3 × 10 mL), and the combined extracts were washed successively with saturated NH_4Cl/concentrated NH_4OH (1:1, 3 × 10 mL), 10% citric acid, aqueous $NaHCO_3$, and brine. The solution was dried over Na_2SO_4, concentrated *in vacuo*, and purified by flash chromatography (acetone/ethyl acetate/hexane = 1/1/1) to obtain **8** (79 mg, 66%) as a white crystalline solid, mp 215−216 °C. TLC: R_f (silica gel, acetone/ethyl acetate/hexane = 1/1/1) = 0.62. ^{1}H NMR (DMSO-d_6): δ 9.51 (1H, t, J = 5.58 Hz), 8.96 (1H, s), 8.42 (1H, s), 7.90−7.95 (2H, m), 7.48−7.62 (3H, m), 4.81 (2H, d, J = 6.00 Hz), 3.45 (3H, s); ^{13}C NMR (DMSO-d_6): δ 171.55, 166.59, 160.93, 157.07, 145.41, 141.08, 133.43, 133.18, 131.67, 128.44, 127.26, 123.07, 51.86, 41.04. Anal. Calcd for $C_{16}H_{13}N_3O_4S$: C, 55.97; H, 3.82; N, 12.24. Found: C, 55.64; H, 3.91; N, 12.39.

Supporting Information Available: ^{13}C NMR spectra for compounds **5** and **6** (4 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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