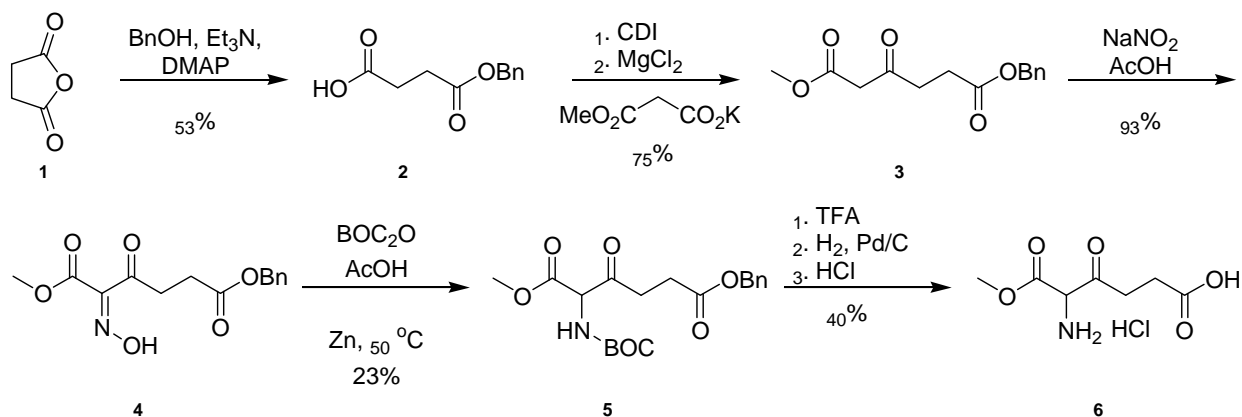


Supplemental Data

Supplemental Experimental Procedures

Synthesis of 5-amino-6-methoxy-4,6-dioxohexanoic acid



4-(Benzyloxy)-4-oxobutanoic acid (**2**). To an ice-cooled solution of benzyl alcohol (3.10 ml, 30.0 mmol) in dichloromethane (Volume: 75 ml) was added triethylamine (6.25 ml, 44.8 mmol) and 4-dimethylaminopyridine (DMAP) (0.15 g, 1.228 mmol) followed by the dropwise addition of succinic anhydride (2.5 g, 24.98 mmol). The reaction mixture was stirred at room temperature overnight and washed with 1N HCl (2×30 mL) and brine (30 mL). The organic layer was dried on sodium sulfate and concentrated in vacuo. On standing, the oily product solidified. The solid was shown to contain an impurity (likely from triethylamine), and thus, the solid was washed with water and hexane, and, lastly, dried under vacuum to give a white solid (2.76 g isolated).

6-Benzyl 1-methyl 3-oxohexanedioate (**3**). A dry flask was charged with 4-(benzyloxy)-4-oxobutanoic acid (1g, 4.80 mmol) and anhydrous THF (Volume: 10.67 ml) under an atmosphere of nitrogen. To the solution was then added 1,1'-carbonyldiimidazole (CDI) (0.935 g, 5.76 mmol) and the resulting mixture was stirred for 1 h at room temperature. At this time, magnesium chloride (0.457 g, 4.80 mmol) and potassium 3-methoxy-3-oxopropanoate (0.750 g, 4.80 mmol) were both added at once. The solution was then stirred overnight at 35 °C (oil bath). The resulting white slurry was filtered through celite, rinsed with THF, and the filtrate was concentrated in *vacuo*. The residue was then

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diluted with ethyl acetate (EtOAc) and washed sequentially with 1M HCl, saturated NaHCO₃, and brine. The organic fraction was dried over Na₂SO₄. ¹H-NMR and LC/MS showed mostly the expected product with a minor impurity. The material was used without further purification. ¹H-NMR (CDCl₃): 7.35 (5H, m), 5.12 (2H, s), 3.73 (3H, s), 3.50 (2H, s), 2.88 (2H, t, *J* = 6.5 Hz), 2.68 (2H, t, *J* = 6.5 Hz). LC/MS: 287 (M+23).

6-Benzyl 1-methyl 2-(hydroxyimino)-3-oxohexanedioate (4). A flask was charged with 6-benzyl 1-methyl 3-oxohexanedioate (958 mg, 3.63 mmol) in acetic acid (14 ml) and cooled in an ice water bath (the solution solidified initially, it was then allowed to warm to the point of stirring). Once cooled, a solution of sodium nitrite (375 mg, 5.44 mmol) in water (7 ml) was added dropwise (slowly over ~10 min, keeping the temperature below 10 °C). The solution was then allowed to slowly warm to room temperature in the ice water bath. Upon completion (by LC/MS), the solution was diluted with EtOAc and washed twice with water and twice with saturated NaHCO₃. The organic portion was dried over Na₂SO₄ and concentrated in vacuo to give 6-benzyl 1-methyl 2-(hydroxyimino)-3-oxohexanedioate (986 mg, 3.36 mmol, 93% yield). ¹H-NMR showed full conversion with trace impurities. The material was used without further purification. ¹H NMR (500 MHz, CDCl₃) δ 7.44 – 7.28 (m, 5H), 5.13 (s, 2H), 3.90 (s, 2H), 3.16 (t, *J* = 6.7 Hz, 2H), 2.72 (t, *J* = 6.8 Hz, 2H). LC/MS: 316.0 (M+23).

6-benzyl 1-methyl 2-((tert-butoxycarbonyl)amino)-3-oxohexanedioate (5). A round bottom flask was charged with 6-benzyl 1-methyl 2-(hydroxyimino)-3-oxohexanedioate (0.5 g, 1.705 mmol) in acetic acid (20 ml) and cooled to 0 °C. To this solution was then added BOC₂O (3.72 g, 17.05 mmol), followed by the portion-wise addition of zinc dust (1.115 g, 17.05 mmol). The reaction mixture was then warmed to 50 °C in an oil bath. LC/MS after 1 h showed complete consumption of the starting material and the solution was cooled to room temperature. Water was then added and the mixture was filtered through celite, washing with dichloromethane. The organic portion was then washed with saturated NaHCO₃ and dried over Na₂SO₄. The residue was purified on a 50 g SiO₂ column using a hexane/EtOAc gradient (15% to 30%). The clean fractions were combined to give 149 mg (23%) of the

expected product (additional product was present in mixed fractions). ^1H NMR (500 MHz, CDCl_3) δ 7.43 – 7.28 (m, 5H), 5.72 (d, $J = 6.9$ Hz, 1H), 5.12 (s, 1H), 5.10 (s, 2H), 3.80 (s, 3H), 2.88 (t, $J = 6.5$ Hz, 1H), 2.74 – 2.64 (m, 3H), 1.44 (s, 9H). LCMS: 402.1 (M+23).

5-Amino-6-methoxy-4,6-dioxohexanoic acid hydrochloride (6). A vial was charged with 6-benzyl 1-methyl 2-((tert-butoxycarbonyl)amino)-3-oxohexanedioate (75 mg, 0.198 mmol) and CH_2Cl_2 (1.5 ml). To this solution was then added TFA (0.5 ml) and the mixture was stirred until deprotection was complete by LCMS (~30 min). The reaction was then concentrated in *vacuo*. ^1H NMR and LC/MS showed only minor impurities so the material was carried forth in the next step. A round bottom flask was charged with 5% Pd/C (30 mg, 0.014 mmol) under an atmosphere of nitrogen. To this was then added methanol (3 ml) and 6-benzyl 1-methyl 2-amino-3-oxohexanedioate (55.3 mg, 0.198 mmol). A hydrogen-filled balloon was then affixed to the reaction vessel and the flask was evacuated then purged with hydrogen three times. The mixture was then vigorously stirred at room temperature. LC/MS after 1 h showed complete cleavage of the benzyl ester. The mixture was filtered through celite, washing with additional methanol. Concentration gave 73 mg of crude product. To this residue was then added 4N HCl/dioxane and diethylether. Partial concentration in *vacuo* resulted in the formation of a white solid which was shown to be the clean hydrochloride salt. Filtration and concentration in *vacuo* gave 18 mg (40%) of **6**. ^1H NMR (500 MHz, D_2O) δ 3.95 (s, 3H), 3.39 – 3.25 (m, 1H), 3.11 – 2.97 (m, 1H), 2.75 (m, 2H). The methine peak is obscured by the D_2O peak, but it appears at 5.38 (s, 1H) in $\text{DMSO-}d_6$ (though DMSO obscures the methylene peaks). LC/MS: 190.0 (M+1).