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Improved Synthesis of a Cyclic Glutamine Analogue Used in Antiviral Agents Targeting 3C and 3CL Proteases Including SARS-CoV-2 M^{pro}

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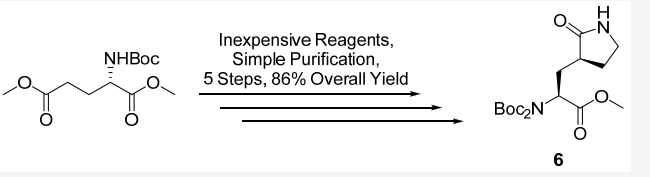
ABSTRACT: An intermediate in the synthesis of numerous antiviral protease inhibitors is the glutamine analogue, (3*S*)-pyrrolid-2-one-3-yl-L-alanine. Preparations of compounds based on this pharmacophore are hindered by the lack of a reliably high yielding synthesis of protected forms of this amino acid. We describe an improved scalable route with readily available reagents and facile purification. This methodology employs γ-allylation of dimethyl *N*-BocGlu, further Boc N-protection, OsO_4-periodate oxidation, O-Me oxime formation, and RaNi-catalyzed hydrogenolysis with concomitant cyclization under basic conditions.



Many positive strand RNA viruses of the *Picornaviridae* and *Coronaviridae* families require a cysteine protease, typically designated as 3C or 3CL, to specifically process an initially synthesized protein through cleavage at glutamine (Gln) residues.[1−5] This process produces the protein fragments essential for viral replication. Well known picornaviruses include poliovirus, human rhinovirus, and foot and mouth disease virus.[1] The coronavirus group has many animal and human pathogens, notably the severe acute respiratory syndrome 2 (SARS-CoV-2) virus that causes COVID19.[4−8] Inhibition of its main chymotrypsin-like cysteine (3CL) protease, known as M^{pro}, is a key objective for development of drugs to combat this disease.[2,6,8−18] A key building block for many such protease inhibitors (Figure 1) is the cyclic glutamine analogue, (3*S*)-pyrrolid-2-one-3-yl-L-alanine (**1**).[2,6,8,9,11,12,14,17−20]

Its constrained structure binds well to viral protease residues required for recognition of the cleavage site. It also prevents interaction of the side chain amide moiety with electrophilic groups intended to interact with the cysteine thiol of the target protein. Reported yields for the synthesis of this building block have varied, typically from 27% to 66% overall yield[19,21−23,20,24] starting from *N*-Boc-L-glutamate dimethyl ester (Scheme 1). The original kilogram-scale synthesis developed by Pfizer Global Research and Development reported an overall yield of 40% over 3 steps[19]—a testament to the difficulties associated with this seemingly facile process.

Despite some reports of higher yields,[24] in our hands, ~40% is generally a maximum. The protected (3*S*)-pyrrolid-2-one-3-yl-L-alanine is commercially available from some fine chemical suppliers, typically at a cost of ca. $150 for 50 mg. In the present work, we report a slightly longer (5 steps), but higher yielding, procedure to generate this amino acid in suitably protected form that requires minimal purification and appears easily scalable to make larger quantities.

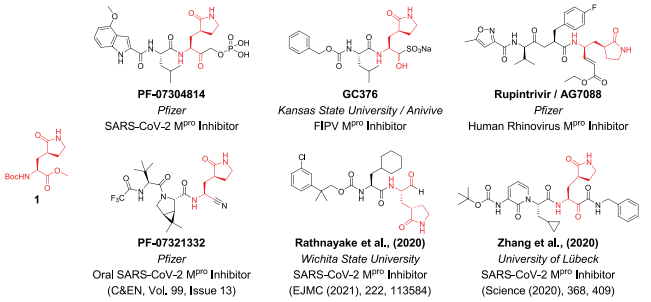
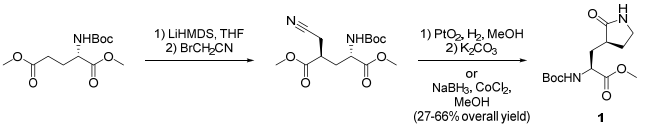


Figure 1. Select viral inhibitors containing a cyclic glutamine analogue, incorporated via utilization of compound **1**. Substructures in inhibitors derived from compound **1** are denoted in red.

Scheme 1. Standard Synthesis of Glutamine Analogue 1



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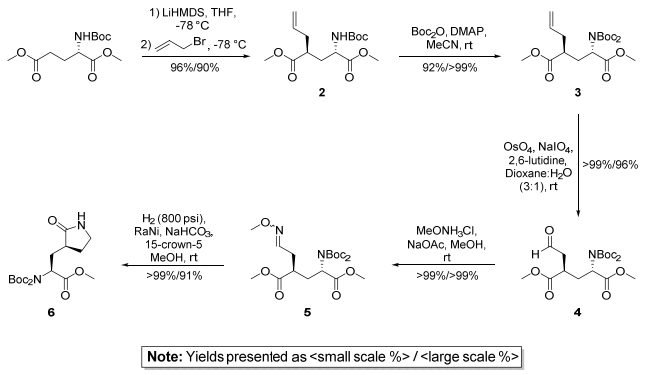
The previously reported synthetic pathway in Scheme 1 begins with the stereoselective cyanomethylation of the γ-carbon of Boc-L-glutamate dimethyl ester. This method was initially developed by Hanessian and co-workers[25,26] and later modified by Tian et al.[19] This is followed by a reduction of the cyano group to a primary amine via hydrogenation in the presence of PtO_2 or borohydride reduction utilizing $NaBH_4$ in the presence of $CoCl_2$. Subsequent cyclization onto the adjacent methyl ester to form the five-membered lactam ring then furnishes the desired compound. A major point of product loss is the initial alkylation step. The Hanessian group had reported that allylation provided much higher yields, especially with allyl bromide,[25] compared to alkylation with bromoacetonitrile. Hence, we developed the route shown in Scheme 2 to make the bis-*N*-Boc protected (3*S*)-pyrrolid-2-one-3-yl-L-alanine methyl ester (**6**).

Scheme 2. Synthesis of Glutamine Analogue 6



Smaller scale (750 mg) synthesis utilizing allyl bromide as previously described[25] gives alkene **2** in 96% isolated yield. Direct oxidative cleavages of the alkene to an aldehyde are low yielding and generate an array of undesired products, as is typical for glutamate and aspartate aldehyde derivatives when there is a single protecting group on the amide nitrogen.[27] Hence, the nitrogen atom was further Boc protected in 92% yield to provide **3**. Although ozonolysis of **3** generates side products, Lemieux–Johnson oxidation with catalytic quantities of OsO_4 (2 mol %) in the presence of $NaIO_4$ and 2,6-lutidine afforded aldehyde **4** in quantitative yield.

At this point, the aldehyde had to be transformed into a primary amine for ring closure. Several approaches were attempted, including direct reductive amination using ammonia, imine formation using benzyl amine, followed by catalytic reduction, and oxime/oxime ether formation, followed by N–O bond cleavage and reduction. The latter proved to be best. Initial use of hydroxylamine hydrochloride formed the desired oxime in modest yields (~70%) with side products, rendering it less suitable for scale-up. As an alternative, *O*-benzylhydroxylamine and *O*-methylhydroxylamine were examined. *O*-Benzylhydroxylamine formed the desired oxime ether in 89% isolated yield, whereas reaction with *O*-methylhydroxylamine gave **5** in quantitative yield. Reduction of the methyl oxime ether **5** was attempted using a number of methods. Reductive cleavage via hydrogenation using Pd/C is a common approach, but generally requires acidic conditions, which are incompatible with the Boc protection. However, Raney Nickel (RaNi) successfully reduced the methyl oxime to a primary amine. Optimization showed that hydrogenolysis at higher pressures using more dilute substrate concentrations afforded cleaner transformation. We hypothesize that this is due to a more rapid reduction combined with diminished intermolecular interactions that could result in dimerization events. Furthermore, RaNi has a distinct advantage in the large scale synthesis because the catalyst can be cleanly removed by employment of a magnetic source.[28] Concurrent with the reduction step, cyclization can be achieved in the presence of $NaHCO_3$ and 15-crown-5 ether, cleanly producing cyclized product **6** in quantitative yield.

We next examined this synthetic sequence on multi-gram scale. Allylation of Boc-glutamic acid dimethyl ester (10 g) gave alkene **2** in somewhat lower 90% yield after purification by flash chromatography. The di-Boc protection proceeded in nearly quantitative yield to furnish **3**, which was efficiently purified via a small silica gel plug. Lemieux–Johnson oxidation of this compound then furnished aldehyde **4** in 96% isolated yield after filtration through a silica plug. Oxime ether **5** was obtained as pure material by extraction and aqueous washing in quantitative yield. Hydrogenolysis of **5** with RaNi gave glutamine analogue **6** in 91% yield after a rapid purification by flash chromatography. To confirm the utility of **6** as an alternative to the mono-Boc derivative **1**, it was deprotected with TFA and directly coupled to *N*-Cbz-Leu-OH to form the corresponding known[7−9] dipeptide **7** in 89% isolated yield. As expected, the process proceeds in comparable yield.

In summary, an improved synthetic route is described to a protected derivative **6** of (3*S*)-pyrrolid-2-one-3-yl-L-alanine. In comparison to the benchmark 40% overall yield to **1** starting with Boc-glutamic acid dimethyl ester, the synthetic route presented here reliably furnishes **6** in 79–86% overall yield with minimal purification. It employs inexpensive, readily available reagents with the use of only catalytic quantities of more valuable transition metals (e.g., OsO_4). With the widespread utilization of this glutamine pharmacophore in numerous antiviral compounds, this route can facilitate production of drug candidates that bind to viral cysteine proteases and expedite efforts toward the design of new antivirals.

EXPERIMENTAL SECTION

Product Characterization. Nuclear magnetic resonance (NMR) spectra were obtained using an Agilent VNMRS 700 MHz or Agilent/Varian VNMRS 500 MHz spectrometers. For ^1H spectra, δ values were referenced to $CDCl_3$ (7.26 ppm), and for ^13C spectra, δ values were referenced to $CDCl_3$ (77.16 ppm) the solvent. Signal assignments were confirmed using 2D NMR methods including COSY, HSQC, and HMBC as required. Infrared spectra (IR) were recorded on a Nicolet Magna 750 or a 20SX FT-IR spectrometer. Cast film and thin film refer to the evaporation of a solution on a NaCl plate. Mass spectra were recorded on a ZabSpec IsoMass VG (high-resolution electrospray (ES)). LC-MS analysis was performed on an Agilent Technologies 6220 orthogonal acceleration TOF instrument equipped with +ve and −ve ion ESI ionization, and full-scan MS (high-resolution analysis) with two-point lock mass correction operating mode. The instrument inlet was an Agilent Technologies 1200 SL HPLC system.

Reagents and Solvents. All commercially available reagents and protected amino acids were purchased and used without further purification unless otherwise noted. All the solvents used for reactions were used without further purification unless otherwise noted. Dry solvents refer to solvents freshly distilled over appropriate drying reagents prior to use. Commercially available ACS grade solvents (>99.0% purity) were used for column chromatography without any further purification.

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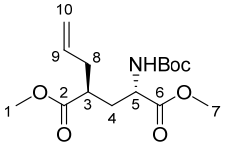
Purification. All reactions and fractions from column chromatography were monitored by thin layer chromatography (TLC) using glass plates (5 × 2.5 cm) precoated (0.25 mm) with silica gel (normal SiO_2, Merck 60 F254). Visualization of TLC plates was performed by UV fluorescence at 254 nm in addition to staining by KMnO4. Flash chromatography was performed using Merck type 60, 230−400 mesh silica gel at elevated pressures.

Synthesis. *Dimethyl (2S,4S)-2-Allyl-4-((tert-butoxycarbonyl)-amino)pentanedioate (**2**).*



2

Small Scale. This procedure was adapted from the literature.[25] Commercially available Boc-L-glutamate dimethyl ester (0.750 g, 2.74 mmol, 1.0 equiv) was dissolved in 11.0 mL of freshly distilled THF in a flame-dried 50 mL round-bottom flask under an argon atmosphere. The solution was then cooled to −78 °C, and LiHMDS (1 M in THF, 5.92 mL, 5.92 mmol, 2.16 equiv) was added dropwise over 5 min. The reaction mixture was then allowed to incubate at −78 °C for an additional 1 h. Allyl bromide (0.710 mL, 8.22 mmol, 3.0 equiv) was then added dropwise over a period of 30 min, and the reaction mixture became a light yellow color. The reaction mixture was checked by TLC after 2 h, and the starting material was found to be fully consumed. The reaction was quenched via addition of HCl (1 M, 7.5 mL) and subsequently extracted with EtOAc (3×). The combined EtOAc layers were washed with H_2O (2×) and brine (1×). The organic layer was then dried over $MgSO_4$, filtered, and concentrated to furnish a light yellow oil as a crude. The material was then purified by flash chromatography over silica, using an eluent system of 15/85 EtOAc/Hexane. Product elution was monitored by $KMnO_4$ staining (R_f = 0.44, 30/70 EtOAc/Hexane). Concentration of product fractions furnishes a clear, slightly yellow oil as the desired compound (0.830 g, 2.63 mmol, 96% yield).

Large Scale. Commercially available Boc-L-glutamate dimethyl ester (10.00 g, 36.32 mmol, 1.0 equiv) was dissolved in 134.5 mL of freshly distilled THF under an argon atmosphere in a flame-dried 500 mL round-bottom flask. The reaction mixture was then cooled to −78 °C, and LiHMDS (1 M in THF, 79.90 mL, 79.90 mmol, 2.2 equiv) was added dropwise. The reaction mixture was then allowed to stir at −78 °C for 1 h. Next, allyl bromide (9.42 mL, 109 mmol, 3.0 equiv) was added dropwise over a period of 15 min. The reaction mixture was then stirred at −78 °C for 4 h, and a check by TLC showed minimal starting material remaining at the end of this period. The reaction mixture was then diluted with 75 mL of sat. $NH_4Cl_{(aq)}$ to quench the reaction. The reaction mixture was then warmed to rt and diluted with 75 mL of EtOAc. The layers were separated, and the aqueous layer was then extracted with additional EtOAc (2×). The combined EtOAc layers were washed with H_2O (2×) and brine (1×), then dried over $MgSO_4$. Filtration and subsequent concentration of the organic layer then furnish a yellow oil as a crude product. The material was purified by flash chromatography over silica using an eluent system of 15/85 EtOAc/Hexane. Product elution was monitored by $KMnO_4$ (R_f = 0.15, 15/85 EtOAc/Hexane). Concentration of product fractions furnishes a light yellow oil as the desired product (10.34 g, 32.78 mmol, 90% yield).

IR (DCM cast film, ν_{max}/cm^{-1}) 3370, 3080, 2979, 2954, 1739, 1718, 1516, 1440, 1169.

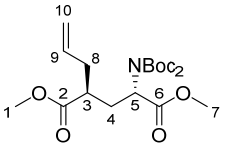
^{1}H NMR (700 MHz, $CDCl_3$) δ_H 5.75−5.65 (1H, m, H9), 5.11−5.02 (2H, m, H10), 4.95 (1H, d, NH), 4.41−4.29 (1H, m, H5), 3.72 (3H, s, H7), 3.66 (3H, s, H1), 2.57 (1H, app quint, H3), 2.41−2.27 (2H, m, H4), 2.00 (2H, app t, H8), 1.44 (9H, s, 3× Boc-CH_3).

^{13}C NMR{1H} (175 MHz, $CDCl_3$) δ_c 175.5 (C2), 172.9 (C6), 155.4 (Boc-C═O), 134.4 (C9), 117.7 (C10), 80.1 (Boc-4 °C), 52.4 (C7), 52.2 (C5), 51.8 (C1), 41.9 (C3), 36.5 (C4), 33.8 (C8), 28.3 (Boc-CH_3).

OR $[\alpha]_D^{26}$ = +74.42 (c = 0.23, DCM).

HRMS (ESI) Calcd for $C_{15}H_{25}NNaO_6$ $[M + Na]^+$ 338.1574, found 338.1570.

*Dimethyl (2S,4S)-2-Allyl-4-(bis(tert-butoxycarbonyl)amino)-pentanedioate (**3**).*



3

Small Scale. This procedure was adapted from the literature.[27,29] Compound **2** (3.00 g, 9.51 mmol, 1.0 equiv) was dissolved in 31.7 mL of MeCN in a flame-dried 100 mL round-bottom flask. To this were successively added DMAP (0.230 g, 1.90 mmol, 0.2 equiv) and Boc_2O (8.30 g, 38.1 mmol, 4.0 equiv). The reaction mixture then quickly changed color to a light orange and was allowed to stir at rt. A check by TLC at 94 h showed near total consumption of starting material. The reaction mixture was then concentrated in vacuo to ca. 10 mL to remove the MeCN. After concentration to a minimal volume, the material was partitioned between ca. 75 mL each of EtOAc and 1 M citric acid, then separated. The EtOAc layer was washed once more with H_2O, then once with brine. The EtOAc layer was then dried over Na_2SO_4 and concentrated to furnish a crude, dark orange oil. This material was purified by flash chromatography using an eluent system of 15/85 EtOAc/Hexane. Product elution was monitored by TLC and $KMnO_4$ staining (R_f = 0.29, 15/85 EtOAc/Hexane). Concentration of product fractions furnishes a yellow oil as the desired product (3.63 g, 8.74 mmol, 92% yield).

Large Scale. Compound **2** (9.500 g, 30.12 mmol, 1.0 equiv) was deposited in a flame-dried 250 mL round-bottom flask and dissolved in 100 mL of freshly distilled MeCN. DMAP (0.740 g, 6.02 mmol, 0.2 equiv) and Boc_2O (26.30 g, 120.5 mmol, 4.0 equiv) were then sequentially added under an argon atmosphere. The reaction mixture was then capped under an argon atmosphere and allowed to stir at rt, changing to a yellow color. After 99 h, a TLC check of the reaction mixture showed total consumption of starting material. The MeCN was removed by concentrating the reaction mixture in vacuo to a minimal volume (ca. 30 mL) and was then partitioned between ca. 250 mL each of EtOAc and 1 M citric acid. The layers were separated, and the aqueous layer was then extracted with additional EtOAc (2×). The combined EtOAc layers were then washed with H_2O (1×) and brine (1×), then dried over Na_2SO_4. Concentration in vacuo then furnishes a dark yellow oil. A TLC of this crude material showed only product (R_f = 0.29, 15/85 EtOAc/Hexane, $KMnO_4$ staining), along with a spot on the baseline. The crude oil (crude mass ca. 15 g) was deposited onto a silica plug (silica mass ca. 135 g) and eluted with 1.25 L of eluent (15/85 EtOAc/Hexane). Total elution was monitored by TLC. Concentration of product fractions furnished a yellow oil as the desired product (12.51 g, 30.12 mmol, >99% yield).

IR (DCM cast film, ν_{max}/cm^{-1}) 3079, 2981, 2953, 1715, 1717, 1703, 1369, 1167, 1145.

^{1}H NMR (700 MHz, $CDCl_3$) δ_H 5.73 (1H, ddt, J = 16.9, 9.9, 7.0 Hz, H9), 5.07 (1H, dq, J = 17.2, 1.6 Hz, H10), 5.05−5.01 (1H, m, H10), 4.96 (1H, dd, J = 8.9, 5.5 Hz, H5), 3.71 (3H, s, H7), 3.65 (3H, s, H1), 2.59 (1H, ttt, J = 14.1, 8.26, 5.9 Hz, H3), 2.39−2.33 (1H, m, H4), 2.31−2.23 (3H, m, H4, H8), 1.50 (18H, s, 6× Boc-CH_3).

^{13}C NMR{1H} (175 MHz, $CDCl_3$) δ_c 175.4 (C2), 171.1 (C6), 151.8 (Boc-C═O), 134.9 (C9), 117.4 (C10), 83.3 (Boc-4 °C), 56.6 (C5), 52.3 (C7), 51.7 (C1), 42.8 (C3), 36.7 (C4), 32.2 (C8), 28.0 (Boc-CH_3).

OR $[\alpha]_D^{26}$ = −21.81 (c = 0.54, DCM).

HRMS (ESI) Calcd for $C_{20}H_{33}NNaO_8$ $[M + Na]^+$ 438.2098, found 438.2091.

https://doi.org/10.1021/acs.joc.1c01299
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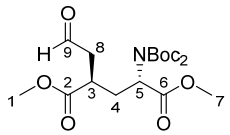
*Dimethyl (2S,4R)-2-(Bis(tert-butoxycarbonyl)amino)-4-(2-oxoethyl)pentanedioate (**4**).*



4

Small Scale. This procedure was adapted from the literature.[30] Compound **3** (0.166 g, 0.400 mmol, 1.0 equiv) was dissolved in a mixture of dioxane/H_2O (3:1, 4.00 mL) in a 25 mL round-bottom flask. To this solution were added 2,6-lutidine (0.093 mL, 0.800 mmol, 2.0 equiv), OsO_4 (0.002 g, 0.008 mmol, 0.02 equiv), and $NaIO_4$ (0.342 g, 1.60 mmol, 4.0 equiv) in succession. The reaction mixture was then capped with a septa and allowed to stir at rt, quickly turning into a slurry. After 2 h, the reaction mixture was checked by TLC 30/70 EtOAc/Hexane), showing consumption of all starting material. The reaction mixture was then diluted with ca. 25 mL each of DCM and H_2O, then separated. The H_2O layer was further extracted with DCM (2×), and the combined DCM layers were washed with brine (1×). The DCM layer was dried over Na_2SO_4 and concentrated to furnish a crude that turned black in coloration. The crude material was purified via flash chromatography using an eluent system of 20/80 EtOAc/Hexane. The black impurity was found to stick to the baseline of the silica. Product elution was monitored by TLC and $KMnO_4$ staining (R_f = 0.29, 30/70 EtOAc/Hexane). Concentration of product fractions and co-evaporation with Et_2O furnishes an oil (0.167 g, 0.400 mmol, >99% yield).

Large Scale. Compound **3** (12.00 g, 28.88 mmol, 1.0 equiv) was dissolved in a mixture of dioxane/H_2O (3:1, 290 mL) in a 500 mL round-bottom flask. To this solution were added 2,6-lutidine (6.69 mL, 57.8 mmol, 2.0 equiv), OsO_4 (0.147 g, 0.578 mmol, 0.02 equiv), and $NaIO_4$ (24.71 g, 115.5 mmol, 4.0 equiv) in succession, and the reaction mixture was capped with a septa. A check by TLC (30/70 EtOAc/Hexane) after 3 h showed complete consumption of starting material. The reaction mixture was then diluted with ca. 1 L each of DCM and H_2O, and the layers were separated. The H_2O layer was further extracted with DCM (2×), and the combined DCM layers were washed with brine. Drying over Na_2SO_4 and concentration furnished a crude dark brown oil. The crude material (crude mass ca. 25 g) was loaded onto a silica plug (silica mass ca. 130 g) and eluted using 1.4 L of 15/85 EtOAc/Hexane, with elution completion being confirmed by TLC (R_f = 0.29, 30/70 EtOAc/Hexane). Concentration of product solution furnishes a brown-black oil after co-evaporation with Et_2O (11.57 g, 27.71 mmol, 96% yield).

IR (DCM cast film, ν_{max}/cm^{-1}) 2981, 2945, 1795, 1743, 1703, 1437, 1369, 1251, 1170, 1146, 1122.

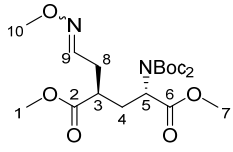
^{1}H NMR (700 MHz, $CDCl_3$) δ_H 9.74 (1H, s, H9), 4.99 (1H, dd, *J* = 9.3, 5.5 Hz, H5), 3.71 (3H, s, H7), 3.69 (3H, s, H1), 2.99−2.94 (1H, m, H3), 2.88 (1H, ddd, *J* = 18.1, 9.1, 1.1 Hz, H8), 2.71 (1H, dd, *J* = 18.1, 4.4 Hz, H8), 2.36−2.30 (1H, m, H4), 2.29−2.24 (1H, m, H4), 1.49 (18H, s, 6× Boc-CH_3).

^{13}C NMR{1H} (175 MHz, $CDCl_3$) δ_c 199.0 (by HSQC, C9), 174.7 (C2), 170.8 (C6), 151.9 (Boc-C═O), 83.6 (Boc-4 °C), 55.9 (C5), 52.4 (C7), 52.2 (C1), 45.1 (C8), 36.4 (C3), 32.0 (C4), 28.0 (Boc-CH_3).

OR $[\alpha]_D^{26}$ = −25.54 (*c* = 0.40, DCM).

HRMS (ESI) Calcd for $C_{19}H_{31}NNaO_9$ $[M + Na]^+$ 440.1891, found 440.1888.

*Dimethyl (2S,4R)-2-(Bis(tert-butoxycarbonyl)amino)-4-(2-(methoxyimino)ethyl)pentanedioate (**5**).*



5

Small Scale. Compound **4** (0.050 g, 0.120 mmol, 1.0 equiv), MeO-NH_2·HCl (0.012 g, 0.144 mmol, 1.2 equiv), and NaOAc (0.020 g, 0.240 mmol, 2.0 equiv) were charged into a 5 mL round-bottom flask, to which dry DCM (0.3 mL) was added. The flask was capped and stirred at rt for 16 h. A check by TLC (25/75 EtOAc/Hexane) at this point showed total consumption of starting material. The reaction mixture was then quenched by addition of ca. 5 mL of sat. $NaHCO_3$, followed by dilution with ca. 5 mL of EtOAc. The layers were then separated, and the aqueous layer was further extracted with EtOAc (2×). The combined EtOAc layers were washed sequentially with sat. $NaHCO_3$ (2×), H_2O (2×), and brine (1×), then dried over Na_2SO_4. Concentration of the EtOAc solution then furnishes a yellow oil as a crude. The material was found to not require further purification (R_f = 0.28, 25/75 EtOAc/Hexane). Concentration of product fractions furnishes a clear, colorless oil as the desired product (mixture of imine isomers, 0.054 g, 0.120 mmol, >99% yield).

Large Scale. Compound **4** (4.18 g, 10.00 mmol, 1.0 equiv), MeO-NH_2·HCl (1.00 g, 12.00 mmol, 1.2 equiv), and NaOAc (1.64 g, 20.00 mmol, 2.0 equiv) were added to a 50 mL round-bottom flask and dissolved with freshly distilled DCM (11.7 mL). The vessel was then capped with a septa and stirred at rt for 16 h. A check by TLC (25/75 EtOAc/Hexane) showed total consumption of starting material, so the reaction mixture was diluted with ca. 150 mL each of EtOAc and sat. $NaHCO_3$, then separated. The aqueous layer was further extracted with EtOAc (2×). The combined EtOAc layers were then washed with sat. $NaHCO_3$ (2×), H_2O (2×), and brine (1×). The EtOAc layer was then dried over Na_2SO_4 and concentrated to furnish a yellow oil (4.47 g, 10.00 mmol, >99% yield). A check of this crude material by NMR showed that it is quite clean and suitable for subsequent use without further purification.

IR (DCM cast film, ν_{max}/cm^{-1}) 2981, 2953, 1797, 1744, 1437, 1369, 1274, 1169, 1146.

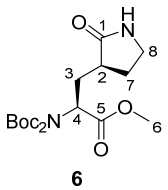
^{1}H NMR (700 MHz, $CDCl_3$) δ_H 7.33 (0.6H, app t, *J* = 6.0 Hz, H9, Isomer A), 6.67 (0.4H, app t, *J* = 5.4 Hz, H9, Isomer B), 5.02−4.97 (1H, m, H5), 3.86 (1.3H, s, H10, Isomer B), 3.78 (1.7H, s, H10, Isomer A), 3.71 (3H, s, H7), 3.68 (1.3H, s, H1, Isomer B), 3.67 (1.7H, s, H1, Isomer A), 2.78−2.69 (1H, m, H3), 2.68−2.39 (2H, m, H8?), 2.36−2.23 (2H, m, H4), 1.50 (18H, s, 6× Boc-CH_3).

^{13}C NMR{1H} (175 MHz, $CDCl_3$) δ_c 174.7 (C2, Isomer B), 174.7 (C2, Isomer A), 170.9 (C6, Isomer B), 170.9 (C6, Isomer A), 151.7 (Boc-C═O), 148.0 (C9, Isomer B), 147.4 (C9, Isomer A), 83.6 (Boc-4 °C), 61.8 (C10, Isomer B), 61.5 (C10, Isomer A), 56.4 (C5, Isomer B), 56.3 (C5 Isomer A), 52.3 (C7), 52.1 (C1, Isomer B), 52.0 (C1, Isomer A), 40.6 (C3, Isomer A), 40.2 (C3, Isomer B), 32.3 (C4, Isomer B), 32.2 (C4, Isomer A), 31.9 (C8, Isomer A), 28.1 (C8, Isomer B), 28.0 (Boc-CH_3).

OR $[\alpha]_D^{26}$ = −20.24 (*c* = 0.99, DCM).

HRMS (ESI) Calcd for $C_{20}H_{34}N_2NaO_9$ $[M + Na]^+$ 469.2157, found 469.2153.

*Methyl (S)-2-(Bis(tert-butoxycarbonyl)amino)-3-((S)-2-oxopyrrolidin-3-yl)propanoate (**6**).*



6

Small Scale. This procedure was adapted from the literature.[31,32] Compound **5** (0.080 g, 0.179 mmol, 1.0 equiv), Raney Nickel (W.R. Grace and Co. Raney 2800, ca. 0.080 g), $NaHCO_3$ (saturated solution at 21 °C, 0.16 mL, ca. 1 equiv), 15-crown-5 ether (0.036 mL, 0.179 mmol, 1.0 equiv), and MeOH (7.0 mL) were added to a vial (Chemglass CG4907-02) with a stir bar and custom cap equipped with a glass frit. The mixture was then placed in a high pressure reaction vessel (Parr Series 4750, 200 mL), stirred, and subjected to purge-charge cycles (3×) with H_2. The reaction mixture was then subjected to H_2 (800 psi, 41372 Torr) for 17.5 h. A check of the reaction mixture at this point by LCMS (see Supporting Information)

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showed consumption of starting material. The catalyst was removed using a magnetic source (computer hard-drive magnet), and the reaction mixture was then partitioned between EtOAc and H_2O, and the layers separated. The H_2O layer was further extracted with EtOAc (2×), and the combined EtOAc layers were washed with brine (1×). Drying over Na_2SO_4 and removal of solvent furnish a yellow crude. This material was purified by flash chromatography, using an eluent system of 85/15 EtOAc/Hexane. Product elution was monitored by TLC and $KMnO_4$ staining (R_f = 0.21, 85/15 EtOAc/Hexane, requires prolonged heating to visualize with $KMnO_4$). Concentration of product fractions furnishes a clear, colorless oil (0.069 g, 0.179 mmol, >99% yield).

Large Scale. Compound **5** (6.00 g, 13.4 mmol, 1.0 equiv), Raney Nickel (W.R. Grace and Co. Raney 2800, ca. 6.00 g), $NaHCO_3$ (saturated solution at 21 °C, 12.0 mL, ca. 1 equiv), and MeOH (538 mL) were added to a glass bottle equipped with a fritted cap. This mixture was then placed within a high pressure reaction vessel, stirred, and subjected to purge-charge cycles with H_2 (3×). The reaction mixture was then subjected to high pressure H_2 (800 psi, 41372 Torr). A check of the reaction mixture at 40 h by LCMS indicated the presence of fully reduced, but uncyclized, material. More $NaHCO_3$ (0.79 g, 9.41 mmol, 0.7 equiv) was added to the reaction, at which point intramolecular cyclization was found to proceed. The cyclization event was found to be sluggish at times, possibly as a result of localized effects, but the cyclization rate can be increased by addition of $NaHCO_3$ (up to 2.5 equiv) or by heating to 60 °C with no detrimental effects. The reaction was continually monitored by LCMS, and upon completion, the Raney Nickel was removed using a magnetic source (computer hard-drive magnet) and the solution was diluted with 1.5 L each of EtOAc and H_2O. The layers were separated, and the aqueous layer was further extracted using EtOAc (2×). The combined organic layers were then washed with brine (1×) and dried over Na_2SO_4. Concentration of the organic extracts then furnishes a yellow oil as a crude. The oil was then subjected to flash chromatography, using an eluent system of 85/15 EtOAc/Hexane. Product elution was monitored by TLC and $KMnO_4$ staining (R_f = 0.21, 85/15 EtOAc/Hexane, prolonged heating required for visualization). Concentration of product fractions furnishes an oil (4.72 g, 12.20 mmol, 91% yield).

IR (DCM cast film, ν_{max}/cm^{-1}) 3227, 2980, 2935, 1791, 1749, 1703, 1369, 1274, 1148, 1124.

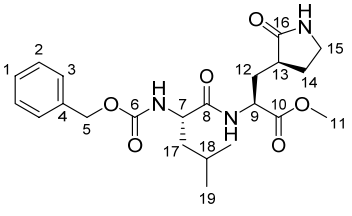
^{1}H NMR (700 MHz, $CDCl_3$) δ_H 5.54 (1H, s, NH), 5.04 (1H, dd, J = 10.2, 4.8 Hz, H4), 3.72 (3H, s, H6), 3.38−3.26 (2H, m, diastereotopic H8), 2.50 (1H, ddd, J = 14.7, 10.1, 3.7 Hz, diastereotopic H3), 2.41−2.32 (2H, m, H2, diastereotopic H7), 2.10 (1H, ddd, J = 14.8, 11.0, 4.8 Hz, diastereotopic H3), 1.88−1.78 (1H, m, diastereotopic H7), 1.51 (18H, s, 6× Boc-CH_3).

^{13}C NMR{1H} (175 MHz, $CDCl_3$) δ_c 179.2 (C1), 171.2 (C5), 152.1 (Boc-C=O), 83.5 (Boc-4 °C), 56.2 (C4), 52.3 (C6), 40.5 (C8), 37.9 (C2), 31.8 (C3), 28.1 (C7), 28.0 (Boc-CH_3).

OR $[\alpha]_D^{26}$ = −30.29 (c = 0.27, DCM).

HRMS (ESI) Calcd for $C_{18}H_{30}N_2NaO_7$ $[M + Na]^+$ 409.1945, found 409.1943.

Methyl (S)-2-((S)-2-(((Benzyloxy)carbonyl)amino)-4-methylpentanamido)-3-((S)-2-oxopyrrolidin-3-yl)propanoate (7).



7

Compound **6** (0.030 g, 0.078 mmol, 1.0 equiv) was dissolved in 2.0 mL of 50/50 DCM/TFA and allowed to deprotect for 1.5 h. The mixture was then concentrated by rotovap and co-evaporated with DCM (5×) to remove residual TFA. This oily material was then dissolved in 0.35 mL of DCM to furnish <Solution 1>.

Cbz-Leu-OH (90% pure, 0.023 g, 0.078 mmol, 1.0 equiv) and HATU (0.030 g, 0.078 mmol, 1.0 equiv) were dissolved in 0.35 mL of DMF. To this were added HOAT (0.6 M solution in DMF, 0.013 mL, 0.008 mmol, 0.1 equiv) and finally DIPEA (0.041 mL, 0.233 mmol, 3.0 equiv). The mixture turned yellow at this point, forming <Solution 2>, and was allowed to incubate at rt for 5 min.

<Solution 1> was then added to <Solution 2>, and the reaction mixture was allowed to stir at rt for 1.5 h. The reaction mixture was then diluted with 7.5 mL each of EtOAc and H_2O. The layers were separated, and the aqueous layer was further extracted with EtOAc (2×). The combined EtOAc layers were washed sequentially with sat. $NaHCO_3$ (2×), 1 M HCl (2×), and brine (1×). Drying over Na_2SO_4, followed by filtration and concentration, furnishes a crude yellow oil. The material was then purified by flash chromatography using EtOAc as the eluent. Product elution was monitored by TLC and $KMnO_4$ staining (R_f = 0.47, 5/95 MeOH/EtOAc). Concentration of product fractions furnishes a transparent oily film as the product (0.030 g, 0.069 mmol, 89%). All characterization data were found to match previously reported values within experimental error.[8]

IR (DCM cast film, ν_{max}/cm^{-1}) 3286, 2956, 2926, 2689, 1537, 1439, 1268.

^{1}H NMR (700 MHz, $CDCl_3$) δ_H 7.88 (1H, d, J = 6.6 Hz, NH), 7.37−7.27 (5H, m, H1, H2, H3), 6.31 (1H, s, NH), 5.44 (1H, d, J = 8.3 Hz, NH), 5.09 (2H, s, H5), 4.50−4.42 (1H, m, H9), 4.39−4.31 (1H, m, H7), 3.72 (3H, s, H11), 3.35−3.23 (2H, m, H15), 2.47−2.30 (2H, m, H13, H14), 2.23−2.14 (1H, m, H12), 1.93−1.85 (1H, m, H12), 1.85−1.77 (1H, m, H14), 1.77−1.70 (1H, m, H18), 1.70−1.63 (1H, m, H17), 1.56−1.47 (1H, m, H17), 1.0−0.91 (6H, m, H19).

^{13}C{^{1}H} NMR (175 MHz, $CDCl_3$) δ_c 179.8 (C16), 171.9 (C8), 172.1 (C10), 152.1 (C6), 136.4 (C4), 128.5 (C2), 128.1 (C3), 128.0 (C1), 66.9 (C5), 53.4 (C7), 52.4 (C11), 51.6 (C9), 42.4 (C17), 40.5 (C15), 38.4 (C13), 32.9 (C12), 28.4 (C14), 24.7 (C18), 22.9 (H19), 22.1 (H19).

OR $[\alpha]_D^{26}$ = −9.89 (c = 0.38, DCM).

HRMS (ESI) Calcd for $C_{22}H_{31}N_3NaO_6$ $[M + Na]^+$ 456.2105, found 456.2102.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.joc.1c01299.

Detailed experimental procedures and spectra of products (PDF)

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Notes

The authors declare no competing financial interest.

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