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Synthesis of Enantiopure ε-Oxapipecolic Acid

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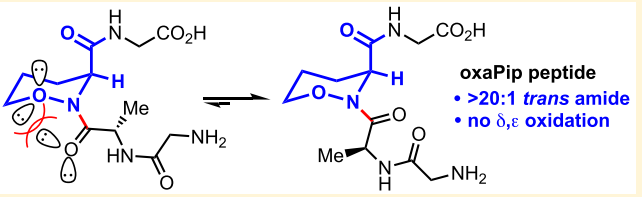
Supporting Information

ABSTRACT: A six-step synthesis of orthogonally protected (*S*)-ε-oxapipecolic acid is described, starting from a commercially available glutamate diester. The approach features *m*CPBA-mediated amine oxidation and an intramolecular Mitsunobu reaction to form the tetrahydrooxazine ring. The enantiopure building block was employed in the synthesis of a short model peptide to determine the amide rotamer preference *N*-terminal to the cyclic residue. In contrast to pipecolic acid, which exhibits a high *cis* amide population, the ε heteroatom in oxapipecolic acid exerts a strong *trans* substantiating effect through lone pair repulsion.



Piperidine-2-carboxylic acid (pipecolic acid; Pip) is a higher homologue of proline (Pro) found in a number of biologically active natural products and peptidomimetics (Figure 1). Rapamycin[1] and FK506,[2] both naturally occurring

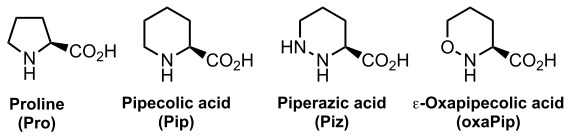


Figure 1. Structures of proline and pipecolic acid derivatives.

macrolides featuring Pip, are potent immunosuppressive agents that bind the peptidyl prolyl *cis/trans* isomerase FKBP12.[3] The *trans* amide geometry at the *N*-terminus of the Pip residue is critical for the binding and biological activity of these and related small molecules.[4] The Pip residue is also encountered in antirheumatic MHC class II ligands,[5] inhibitors of HIV protease,[6] analogues of peptide hormones,[7] as well in the FDA-approved anesthetic ropivacaine.[8] Beyond biomedical applications, Pip derivatives have also been investigated as non-canonical inducers of β-turn geometry within peptides.[9]

Given its close structural relationship to proline, applications of the Pip residue in peptide chemistry have often been guided by studies on the conformational impact of its larger ring size. Principal among these is the often higher *N*-terminal *cis* amide rotamer population observed for Pip relative to Pro resulting from the increased allylic-type strain.[10] Piperazic acid (Piz) is a naturally occurring analogue of Pip featuring CH_2 to NH substitution at the ε position.[11] This nonencoded residue has been shown to exhibit an unusually high *trans* amide rotamer population[12] and is encountered in several bioactive natural products and small molecule drug leads.[12b,13] Our interest in *N*-heteroatom-substituted amino acids led us to consider the oxygen variant of Piz, ε-oxapipecolic acid (oxaPip). Replacement of the ε nitrogen in Piz with oxygen was expected to maintain or promote a strong *trans* amide bias, resulting from enhanced lone pair repulsion in the *cis* conformation (Figure 2).[14] In addition, oxaPip would not suffer from the slow

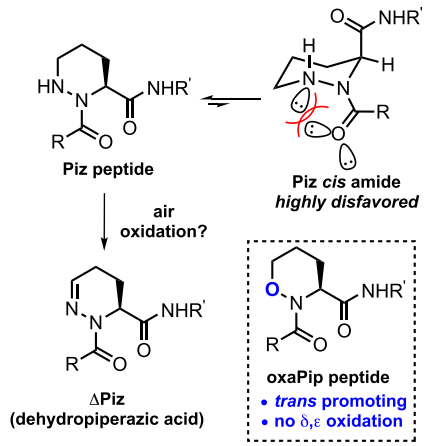


Figure 2. Properties of Piz and oxaPip.

heterocycle oxidation that has been previously observed with Piz.[15] We thus viewed oxaPip as a stable, *trans*-promoting analogue of Pip with potentially widespread applications in peptidomimetic drug design.

Interest in a useful synthetic route toward oxaPip can be traced back to the efforts of Lee and Woodward, who described a tandem malonate nitrosation/annelation reaction to afford the intermediate dihydrooxazine ring.[16] Zimmer,

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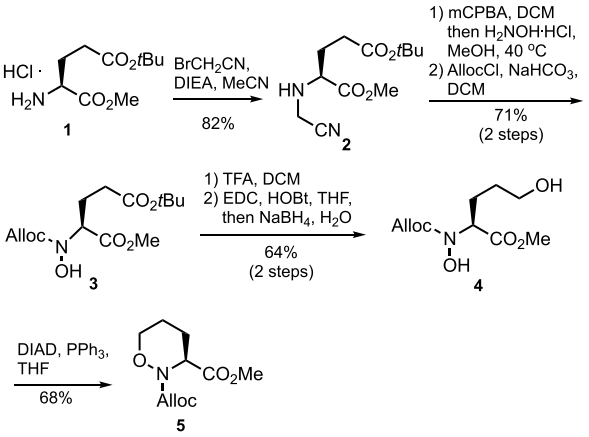
J. Org. Chem. 2020, 85, 1680−1686

Reissig, and co-workers also reported a synthesis of racemic oxaPip ethyl ester via borohydride reduction of a dihydrooxazine.[17] Other routes toward substituted analogues rely primarily on acylnitroso cycloaddition reactions that require the separation of diastereomeric mixtures or enzymatic resolution to provide pure products.[18] A similar strategy was applied to the preparation of analogous δ-oxaproline peptidomimetics harboring additional ring substituents.[18,19] Here, we describe the first synthesis of a protected oxaPip building block suitable for incorporation into host peptides starting from glutamic acid as a chiral synthon.

Our synthesis commenced with the *N*-oxidation of commercially available glutamate derivative **1** according to the procedure developed by Fukuyama and co-workers (Scheme 1).[20] Monoalkylation of **1** with bromoacetonitrile

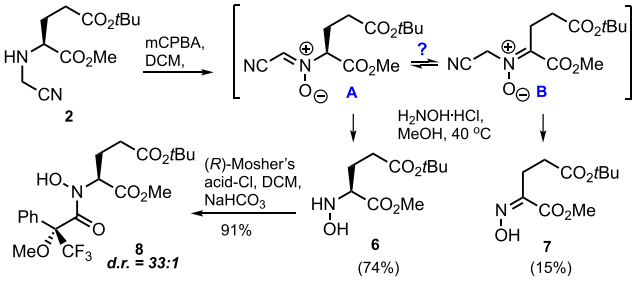
Scheme 1. Synthesis of Alloc-oxaPip-OMe (5)



was thus followed by treatment with mCPBA to give the intermediate nitrone. Aminolysis of the nitrone with hydroxylamine and subsequent Alloc protection afforded compound **3** in 58% yield over 3 steps. The selectivity of the Alloc protection was found to be sensitive to the base and solvent employed, as other conditions led to the appreciable formation of allyl carbonate byproducts. Acidolysis of the *t*-butyl ester in **3** was followed by a reduction of the carboxylic acid via its hydroxybenzotriazole ester with sodium borohydride in a 64% yield. The major byproduct of this reaction was found to be the azalactone resulting from the intramolecular attack of the hydroxyl group onto the side chain HOBt ester. This side product, readily separated from **4** by flash chromatography, was observed under various conditions used to convert the γ-carboxylic acid into an active ester suitable for borohydride reduction. Formation of the tetrahydrooxazine ring was achieved under standard Mitsunobu reaction conditions to provide compound **5** in 68% yield.

In the course of *N*-hydroxylation of compound **2**, we observed a small amount of undesired oxime **7** as a byproduct (Scheme 2). This oxime arises from the aminolysis of regioisomeric nitrone **B** with the hydroxylamine employed in the workup. Although cyanomethylation has previously been shown to favor nitrone regioisomers of type **A**,[20] we considered that the formation **7** might be indicative of equilibration between **A** and **B** under the reaction conditions. If so, the stereochemical integrity of **6** could be compromised, thus severely limiting the utility of building block **5** for peptide synthesis. To test this, we acylated **6** with Mosher's acid

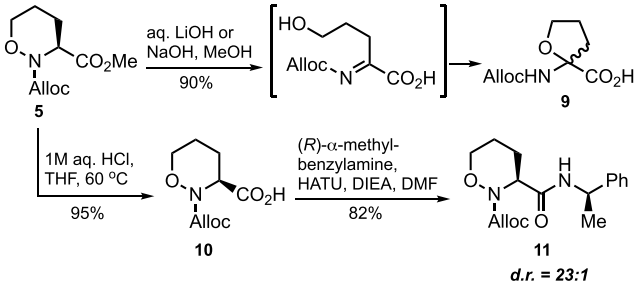
Scheme 2. Enantiopurity of *N*-Hydroxyamino Ester 6



chloride to obtain **8** and compared its ^{1}H NMR spectrum with that of the (*S*,*S*) diastereomer. Integration of well-resolved Hα signals in the crude samples indicated a 33:1 dr for **8**, thus establishing the enantiopurity of **6**. These results suggest nitrone **A** arises from regioselective deprotonation and does not undergo significant equilibration to **B**.

With enantiopure tetrahydrooxazine **5** in hand, we attempted methyl ester hydrolysis under basic conditions, as shown in Scheme 3. Treatment with hydroxide bases resulted

Scheme 3. Synthesis of Alloc-oxaPip−OH (10) and Determination of Diastereomeric Ratio following Amidation



in full conversion to tetrahydrofuran derivative **9**, presumably via elimination to the *N*-acyloxyimine intermediate. However, hydrolysis with aqueous acid provided the desired carboxylic acid **10** in a high yield. To ensure the stereochemical integrity of the α carbon, **10** was converted to (*R*)-α-methylbenzamide derivative **11** with HATU and DIEA. A comparison of the ^{1}H NMR of **11** with that of the diastereomeric amide derivative revealed a 23:1 dr, suggesting a lack of appreciable epimerization during ester hydrolysis or activation of the Alloc-protected building block.

We next prepared *N*-acetyl derivatives of oxaPip and its analogues in order to compare amide rotamer propensities within the series (Table 1).[21] Analysis of the ^{1}H NMR spectra for **12**−**15** in $CDCl_3$ revealed that the ε-heteroatom analogues of Pip (**12** and **13**) were devoid of rotational isomers at rt. This is in sharp contrast to **14** and **15**, which exhibited amide $K_{trans/cis}$ values of 4.0 and 3.6, respectively. The absence of a minor rotamer in the NMR spectrum of **12** leaves open the possibility of conformational averaging due to a low isomerization barrier. However, ^{1}H NMR acquisition at −60 °C did not result in detectable rotameric signals, suggesting true conformational homogeneity at rt.[22] These data are consistent with results previously reported for **13**, wherein Ciufolini and co-workers established the all-*trans* configuration of several acetylated Piz derivatives in a nonpolar solvent.[12] Minor *cis* amide rotamers of **12** and **13** were readily apparent in D_2O.

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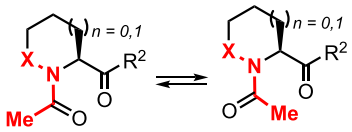
Table 1. *trans*/*cis* Amide Bond Equilibria in 12−15 by ^{1}H NMR at rt

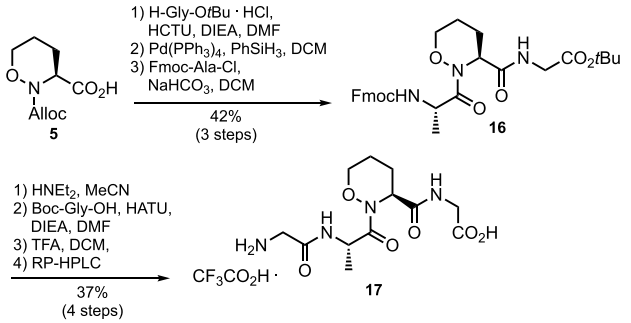


cmpd	X	*n*	residue	$K_{trans/cis}$(CDCl$_3$)	$K_{trans/cis}$(D$_2$O)
12	O	1	Ac-oxaPip-O Me	all *trans*	7.3
13	NH	1	Ac-Piz-O Me	all *trans*	8.9
14	CH_2	1	Ac-Pip-O Me	4.0	2.7
15	CH_2	0	Ac-Pio-O Me	3.6	4.5

The parent pipecolate residue (**14**) again exhibited a much higher fraction of the *cis* rotamer (27%) relative to the ϵ-heteroatom analogues (10−12%). Interestingly, oxaPip derivative **12** exhibited a slightly lower *trans* amide propensity than Piz analogue **13** despite the higher electron density of oxygen. The pronounced increase in *cis* amide population in D$_2$O for **12** and **13** may be due, in part, to the ability of polar solvents to attenuate electrostatic lone pair repulsion.[23]

In order to examine the conformational impact of oxaPip in the context of a peptide, we synthesized a short model sequence for NMR analysis. The Gly-Ala-Xaa-Gly tetrapeptide described by Raleigh and co-workers was previously employed to study the amide rotamer population of both proline and pipecolic acid derivatives by ^{1}H NMR.[10a,24] We thus prepared the variant featuring oxaPip in the Xaa position for comparison. Compound **16** (Scheme 4) was synthesized in

Scheme 4. Synthesis of Model Tetrapeptide 17



solution via amidation of **5** with *t*-butyl glycinate, Alloc removal, and acylation of the crude amine with the preformed acid chloride of Fmoc-protected alanine.[25] This was subjected to Fmoc cleavage with diethylamine, condensation with Boc-protected glycine, and global deprotection with TFA. A portion of crude tetrapeptide **17** was purified by RP-HPLC for NMR analysis.

As shown in Figure 3, two amide conformers were identified in the ^{1}H NMR spectrum of **17**, and the major was assigned *trans* based on NOE correlation between the oxaPip axial Hδ and alanine Hα. The NOESY spectrum also exhibited pronounced exchange between the major Hβ_{eq} and Hα oxoPip signals and their respective well-resolved *cis* rotamer signals.[22] The integration of both pairs of peaks in **17** indicated a $K_{trans/cis}$ of 28.7. We synthesized the previously reported pipecolic acid and proline analogues for direct comparison. In agreement with reported data, these peptides exhibited significantly lower $K_{trans/cis}$ values of 3.8 and 10.5, respectively. The decrease in

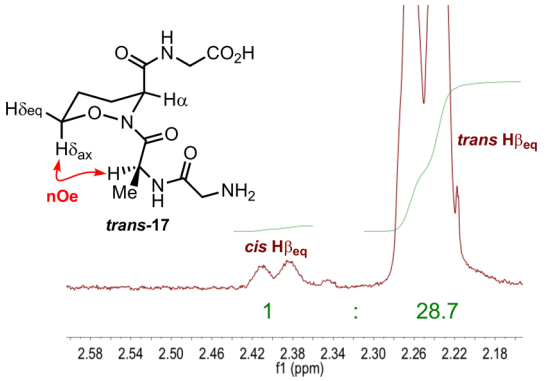


Figure 3. Observed NOE correlation for *trans*-**17** and amide rotamer integrations from 500 MHz ^{1}H NMR (D$_2$O, rt).

the *cis* population of **17** relative to Pip and Pro is consistent with the trend for the monomers in Table 1. Similar conformational characteristics have been observed for acyclic *N*-amino and *N*-hydroxy peptides, as well as derivatives of δ-oxaproline.[14d,26] The 4-fold increase in oxoPip *trans* amide propensity in **17** versus **12** highlights the impact of additional steric and H-bonding interactions present in the tetrapeptide relative to the *N*-acetyl monomer model. Data from **17** thus provides more relevant insight on the conformational preference of oxaPip peptides relative to those harboring Pip.

In summary, we describe the synthesis of enantiopure ϵ-oxapipecolic acid, starting from glutamic acid. Our route relies on regioselective nitrone formation and an intramolecular Mitsunobu reaction to form the tetrahydrooxazine ring. An Alloc-protected oxaPip building block was employed in the synthesis of *N*-acetyl and tetrapeptide models in order to examine the impact of *N*-heteroatom substitution on the amide *cis*/*trans* rotamer population. Like the Piz residue, oxaPip destabilizes the *cis* amide rotamer geometry through apparent lone pair repulsion. However, the absence of an H-bond donor at the ϵ position and the inability of oxaPip to undergo δ,ϵ oxidation[15] distinguishes it from the naturally occurring Piz residue. An investigation into the utility of oxaPip as a constrained Pip surrogate within bioactive peptidomimetics is currently underway in our laboratory.

EXPERIMENTAL SECTION

Synthesis and General Notes. Unless stated otherwise, reactions were performed in flame-dried glassware under a positive pressure of argon or nitrogen gas using dry solvents. Reaction heating was achieved using silicone oil baths. Commercial grade reagents and solvents were used without further purification, except where noted. Toluene, Et$_2$O, DCM DMF, and MeCN were used following passage through the Pure Process Technologies solvent purification system. Other anhydrous solvents were purchased directly from chemical suppliers. Thin-layer chromatography (TLC) was performed using Merck 60 F254 silica gel precoated glass-backed plates (0.25 mm). Flash chromatography was performed using silica gel cartridges (40−65 μm particle size). Reaction progress was judged by TLC analysis (single spot/two solvent systems) using a UV lamp, CAM (ceric ammonium molybdate), ninhydrin, or basic KMnO$_4$ stain(s) for detection purposes. NMR spectra were recorded on a 400 or 500 MHz spectrometer. Proton chemical shifts are reported as δ values relative to residual signals from deuterated solvents (D$_2$O, CDCl$_3$, CD$_3$OD, or DMSO-d_6).

*1-Methyl-5-(tert-butyl)(cyanomethyl)-L-glutamate (**2**).* A mixture of L-Glu(O*t*Bu)-OMe hydrochloride (6.09 g, 24.7 mmol) and DIEA (12.6 mL, 72.2 mmol) in MeCN was treated with bromoacetonitrile

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J. Org. Chem. 2020, 85, 1680−1686

(1.83 mL, 28.9 mmol) dropwise over 10 min at rt. The reaction was stirred for 12 h at 50 °C prior to the removal of MeCN. The residue was dissolved in DCM and washed with sat. aq $NaHCO_3$. The organic layer was collected, and the aq phase was extracted with additional DCM. The combined organic layers were dried over anhydrous $MgSO_4$. Purification by silica gel flash chromatography (30%−40% EtOAc/hexanes) gave **2** as a yellow oil (5.09 g, 82% yield, 94% yield brsm): ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.74 (s, 3H), 3.57 (d, J = 5.4 Hz, 2H), 3.39 (dd, J = 5.3, 7.7 Hz, 1H), 2.12 (bs, 1H), 2.32 (td, J = 4.1, 7.2, 7.0 Hz, 2H), 1.99 (ddd, J = 7.3, 12.7, 14.3 Hz 1H), 1.82 (td, J = 7.4, 14.4 Hz, 1H), 1.41 (s, 9H); ^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$) δ 173.8, 172.1, 117.3, 80.6, 59.4, 52.3, 35.9, 31.4, 28.0, 27.7; $[\alpha]_D^{24}$ −15.7 (c 1.51, $CHCl_3$); HRMS (ESI-TOF) m/z [M + H]$^+$ calcd for $C_{12}H_{21}N_2O_4$257.1502, found 257.1504.

*1-Methyl 5-(tert-Butyl) N-((Allyloxy)carbonyl)-N-hydroxy-L-glutamate (**3**) and 1-Methyl 5-(tert-Butyl) (Z)-2-(Hydroxyimino)-pentanedioate (**6**) and 5-(tert-Butyl) 1-Methyl (Z)-2-(Hydroxyimino)pentanedioate (**7**).* A solution of **2** (3.13 g, 12.2 mmol) in DCM was treated with 70% mCPBA (7.23 g, 29.4 mmol), which was added in two portions over 30 min at 0 °C. After 30 min, the reaction was stirred at rt for 1.5 h. The reaction flask was then cooled to 0 °C prior to the addition of sat. aq $NaHCO_3$ and sat. aq $Na_2S_2O_3$. The resulting slurry was stirred at rt for 30 min until two layers were observed. The organic layer was collected, and the aq phase was extracted with additional DCM. The combined organic layers were dried over anhydrous $MgSO_4$. Concentration on a rotary evaporator provided the crude nitrone intermediate as a white solid. A mixture of the nitrone and hydroxylamine hydrochloride (4.28 g, 61.7 mmol) was dissolved in MeOH then stirred 18 h at 40 °C. The solution was concentrated to remove MeOH. The residue was dissolved in DCM and washed with sat. aq $NaHCO_3$. The organic layer was collected, and the aq phase was extracted with additional DCM. The combined organic layers were dried over anhydrous $MgSO_4$. Purification by silica gel flash chromatography (35%−45% EtOAc/hexanes) gave **6** as a colorless oil (2.11 g, 74% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.58 (bs, 1H), 3.75 (s, 3H), 3.61 (t, J = 7.0 Hz, 1H), 2.32 (td, J = 7.2, 3.2 Hz, 2H), 1.95 (q, J = 7.1 Hz, 2H), 1.42 (s, 9H); ^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$) δ 173.2, 172.7, 80.8, 64.1, 52.2, 31.8, 28.0, 23.6; $[\alpha]_D^{24}$ −12.0 (c 0.75, $CHCl_3$); HRMS (ESI-TOF) m/z [M + H]$^+$ calcd for $C_{10}H_{20}NO_5$ 234.1336, found 234.1326.

In addition to intermediate **6**, a small amount of oxime **7** was obtained as a colorless oil: ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.57 (bs, 1H), 3.76 (s, 3H), 2.78 (m, 2H), 2.43 (m, 2H), 1.34 (s, 9H); ^{13}C{^{1}H} NMR (125 MHz, $CDCl_3$) δ 171.7, 163.8, 151.1, 80.9, 52.6, 31.2, 27.9, 20.4; HRMS (ESI-TOF) (m/z) [M + Na]$^+$ calcd for $C_{10}H_{17}NNaO_5$ 254.0999, found 254.0979.

A mixture of **6** (2.03 g, 8.70 mmol) and $NaHCO_3$ (23.7 g, 43.5 mmol) in DCM was treated with allyl chloroformate (1.39 mL, 13.1 mmol) dropwise over 10 min. After 30 min, an addition 1 equiv of $NaHCO_3$ was added to the reaction mixture. After 1 h, the reaction was quenched with water. The organic layer was collected and the aq phase extracted with additional DCM. The combined organic layers were dried over anhydrous $MgSO_4$. Purification by silica gel flash chromatography (30%- 40%EtOAc/hexanes) gave **3** as a yellow oil (2.64 g, 96% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.03 (bs, 1H), 5.92 (ddd, J = 5.6, 10.8, 22.6 Hz, 1H), 5.33 (d, J = 17.1 Hz, 1H), 5.23 (d, J = 10.4 Hz, 1H), 4.75 (dd, J = 4.7, 10.7 Hz, 1H), 4.67 (d, J = 5.6 Hz, 2H), 3.75 (s, 3H), 2.47 (ddd, J = 4.5, 6.7, 16.3 Hz, 1H), 2.39−2.27 (m, 3H), 1.42 (s, 9H); ^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$) δ 173.9, 170.7, 157.7, 132.0, 118.3, 81.5, 67.1, 61.5, 52.6, 32.1, 28.0, 22.3; $[\alpha]_D^{24}$ −26.1 (c 2.27, $CHCl_3$); HRMS (ESI-TOF) m/z [M + H]$^+$ calcd for $C_{14}H_{24}NO_7$ 318.1554, found 318.1548.

*Methyl-(S)-2-(((allyloxy)carbonyl)(hydroxy)amino)-5-hydroxypentanoate (**4**).* Compound **3** (3.01 g, 9.49 mmol) was treated with a 3:1 mixture of TFA/DCM and stirred at rt for 1 h. The solution was diluted with EtOAc and concentrated. This process was repeated twice to remove the residual TFA, and the resulting oil was dried under a vacuum.

A mixture of the crude carboxylic acid (1.42 g, 5.42 mmol) and 80% HOBt (1.56 mg, 8.13 mmol) dissolved in DCM was treated with EDC (1.56 g, 8.13 mmol), stirred at rt for 30 min, and concentrated on a rotary evaporator. The resulting residue was dissolved in THF and cooled to 0 °C prior to the addition of $NaBH_4$ (615 mg, 16.3 mmol) in water over 10 min. After the addition, the reaction flask was removed from the ice bath to allow the reaction to warm to rt over 15 min. The reaction was diluted with MeOH and EtOAc and then transferred to a separatory funnel. The solution was then washed with 1 M aq HCl and sat. aq $NaHCO_3$ prior to the collection of the organic phase. The aq phases were extracted with additional EtOAc. The combined organic phases were dried over anhydrous $MgSO_4$. Purification by silica gel flash chromatography (70%−80% EtOAc/hexanes) gave **4** as a light yellow oil (847 mg, 64% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.89 (m, 1H), 5.30 (m, 1H), 5.18 (m, 1H), 4.71−4.54 (m, 3H), 3.73−3.63 (m, 4H), 3.58 (m, 1H), 1.98 (m, 2H) 1.65 (m, 2H); ^{13}C{^{1}H} NMR (125 MHz, $CDCl_3$) 171.4, 158.1, 132.1, 118.3, 67.1, 61.9, 61.7, 52.6, 28.6, 24.3; $[\alpha]_D^{24}$ −10.8 (c 0.69, $CHCl_3$); HRMS (ESI-TOF) m/z [M + H]$^+$ calcd for $C_{10}H_{18}NO_6$ 248.1135, found 248.1133

*2-Allyl 3-Methyl-(S)-1,2-oxazine-2,3-dicarboxylate (**5**).* A solution of **4** (234 mg, 945 μmol) and triphenylphosphine (496 mg, 1.89 mmol) in THF was treated with diisopropyl azodicarboxylate (334 μL, 1.70 mmol) dropwise at rt and stirred overnight. The reaction was concentrated to remove THF. Purification by silica gel flash chromatography (80:19:1 $CHCl_3$/hexanes/EtOAc) gave **5** as a clear oil (147 mg, 68% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.95 (ddd, J = 5.7, 10.9, 22.8 Hz, 1H), 5.35 (d, J = 17.2 Hz, 1H), 5.25 (d, J = 10.4 Hz, 1H), 4.86 (d, J = 3.7 Hz, 1H), 4.69 (d, J = 5.5 Hz, 2H), 4.08 (dd, J = 3.9, 11.6 Hz, 1H), 3.92 (m, 1H), 3.77 (s, 3H), 2.36 (dd, J = 6.8, 7.6 Hz, 1H), 2.04−1.74 (m, 2H), 1.59 (m, 1H); ^{13}C{^{1}H} NMR (400 MHz, $CDCl_3$) δ 170.0, 155.6, 132.1, 118.4, 71.9, 66.9, 57.2, 52.6, 23.6, 21.5; $[\alpha]_D^{24}$ −115.0 (c 0.69, $CHCl_3$); HRMS (ESI-TOF) m/z [M + H]$^+$ calcd for $C_{10}H_{16}NO_5$ 230.1029, found 230.1029.

*5-(tert-Butyl) 1-Methyl-hydroxy-N-((R)-3,3,3-trifluoro-2-methoxy-2-phenylpropanoyl)-L-glutamate (**8**).* A solution of (*R*-(+)-α-methoxy-α-trifluoromethylphenylacetic acid (40.0 mg, 170 μmol) in DCM was treated with 1-chloro-*N*,*N*,2-trimethyl-1-propenylamine (34.0 μL, 260 μmol) and stirred for 5 min. This solution was then transferred into a flask containing a mixture of **6** (40.0 mg, 170 μmol) and $NaHCO_3$ (140 mg, 1.7 mmol) dissolved in DCM. This reaction was stirred 18 h and quenched with water. The organic layer was collected and the aq phase extracted with additional DCM. The combined organic layers were dried over anhydrous $MgSO_4$. Purification by silica gel flash chromatography (5%−50% EtOAc/hexanes) gave **8** as a clear oil (70.0 mg, 91% yield): ^{1}H NMR (400 MHz, $CDCl_3$, mixture of rotamers) δ 7.68−7.46 (m, 2H), 7.38 (dd, J = 5.2, 2.0 Hz, 3H), 6.36 (s, 1H), 5.21 (dd, J = 11.2, 4.1 Hz, 0.8H), 4.55 (dd, J = 8.0, 6.0 Hz, 0.2H), 3.93−3.54 (m, 6H), 2.56−2.17 (m, 2.6H), 2.16−2.02 (m, 0.8H), 1.94−1.52 (m, 0.6H), 1.39 (m, 9H); ^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$) δ 172.9, 170.8, 166.7, 133.3, 129.4, 128.9, 128.3, 127.1, 126.6, 84.7, 81.9, 61.3, 58.1, 56.3, 52.8, 32.1, 28.2, 23.2; $[\alpha]_D^{24}$ +3.4 (c 0.36, $CHCl_3$); HRMS (ESI-TOF) m/z [M + H]$^+$ calcd for $C_{20}H_{27}F_3NO_7$ 450.1734, found 450.1745.

*2-(((Allyloxy)carbonyl)amino)tetrahydrofuran-2-carboxylic acid (**9**).* A solution of **5** in a 1:1 mixture of 2 M aq NaOH/methanol was stirred for 30 min at 0 °C. The reaction was acidified to pH 1 with 1 M aq HCl and extracted with EtOAc. The combined organic layers were washed with water and brine and dried over anhydrous $MgSO_4$. Purification by silica gel flash chromatography (0−20% MeOH/$CHCl_3$) gave **9** as a colorless oil (24 mg, 90% yield): ^{1}H NMR (400 MHz, CD_3OD) δ 5.90 (ddt, J = 16.2, 10.6, 5.4 Hz, 1H), 5.29 (d, J = 17.3 Hz, 1H), 5.16 (d, J = 10.6 Hz, 1H), 4.52 (d, J = 5.4 Hz, 2H), 4.11 (d, J = 7.1 Hz, 1H), 3.99 (d, J = 7.1 Hz, 1H), 2.17 (dq, J = 11.6, 7.6, 6.5 Hz, 2H), 2.02 (dt, J = 15.1, 7.1 Hz, 2H); ^{13}C{^{1}H} NMR (101 MHz, CD_3OD) δ 156.3, 132.6, 116.4, 91.9, 68.5, 65.2, 34.8, 29.3, 24.1; HRMS (ESI-TOF) m/z [M + H]$^+$ calcd for $C_9H_{14}N_1O_5$216.0867, found 216.0870.

*(S)-2-((Allyloxy)carbonyl)-1,2-oxazine-3-carboxylic acid (**10**).* A solution of **5** (270 mg, 1.18 mmol) in 10 mL of 1:1 THF/HCl (1 M

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J. Org. Chem. 2020, 85, 1680−1686

aq) was heated to 80° C and stirred overnight. An additional 5 mL of 1 M aq HCl was then added, and the reaction was stirred for an additional 18 h. The reaction was diluted with EtOAc and water. The organic layer was collected and the aq phase extracted with additional EtOAc. The combined organic layers were dried over anhydrous $MgSO_4$. Purification by silica gel flash chromatography (0%−10% $CHCl_3$/MeOH) gave **10** as a clear oil (241 mg, 95% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.07 (bs, 1H), 5.90 (ddd, J = 5.3, 10.5, 22.1 Hz, 1H), 5.54−5.09 (m, 4H), 4.78 (s, 1H), 4.64 (s, 2H), 4.10 (d, J = 10.3 Hz, 1H), 3.87 (t, J = 10.2 Hz, 1H), 2.34 (d, J = 7.7 Hz, 1H), 1.86 (s, 2H), 1.53 (d, J = 7.5 Hz, 1H); ^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$) δ 174.5, 156.4, 132.1, 118.4, 71.9, 67.0, 58.1, 29.7, 23.8, 21.5; $[\alpha]_D^{24}$ −110.0 (c 0.59, $CHCl_3$); HRMS (ESI-TOF) m/z [M + H]$^+$ calcd for $C_9H_{14}NO_5$ 216.0873, found 216.0868.

Allyl (S)-3-(((S)-1-Phenylethyl)carbamoyl)-1,2-oxazine-2-carboxylate (11). A solution of **10** (65 mg, 310 μmol), DIEA (160 μL, 910 μmol), and (*S*)-δ-methyl-benzylamine (78 μL, 610 μmol) in MeCN was treated with HATU (170 mg, 450 μmol) at rt and stirred overnight. The reaction was diluted with EtOAc and quenched with 1 M aq HCl. The organic layer was collected and the aq phase extracted with additional EtOAc. The combined organic layers were dried over anhydrous $MgSO_4$. Purification by silica gel flash chromatography (5%−50% EtOAc/hexanes) gave **11** as a clear oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.28 (m, 5H), 6.77 (d, J = 7.5 Hz, 1H), 5.92 (dq, J = 5.8, 11.0 Hz, 1H), 5.29 (m, 2H), 5.16 (p, J = 7.0 Hz, 1H), 4.75 (d, J = 5.2 Hz, 1H), 4.7 (d, J = 4.8 Hz, 2H), 4.0 (dd J = 3.7, 11.4 Hz, 1H), 3.89 (m, 1H), 2.43 (d J = 12.9 Hz, 1H); ^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$) δ 168.4, 155.7, 143.0, 131.8, 128.8, 127.5, 126.3, 119.2, 71.6, 67.5, 58.0, 49.2, 22.8, 22.0, 21.1; $[\alpha]_D^{24}$ −8.1 (c 1.85, $CHCl_3$);HRMS (ESI-TOF) m/z [M + H]$^+$ calcd for $C_{17}H_{23}N_2O_4$ 319.1652, found 319.1647.

Methyl (S)-2-Acetyl-1,2-oxazinane-3-carboxylate (12). A solution of **5** (74.3 mg, 324 μmol) in DCM was treated with $Pd(PPh_3)_4$ (18.7 mg, 16.2 μmol), phenylsilane (201 μmol, 1.62 mmol), and stirred for 2 h. The volatiles were removed under reduced pressure and the crude material purified via flash chromatography over silica gel (10−60% EtOAc/hexanes). This purified product was immediately dissolved in DCM, treated with acetyl chloride (111 μL, 1.55 mmol) and $NaHCO_3$(261 mg, 3.10 mmol), and stirred for 16 h. The volitiles were removed under reduced pressure, and the crude material was purified via flash chromatography (10−60% EtOAc/hexanes), resulting in a waxy solid (41.2 mg, 68% yield over 2 steps): ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.26 (m, 1H), 4.10 (m, 1H), 3.84 (m, 1H), 3.76 (s, 3H), 2.34 (m, 1H), 2.15 (s, 3H), 1.86 (m, 2H), 1.63 (m, 1H); ^{13}C{^{1}H} NMR (125 MHz, $CDCl_3$) δ 171.0, 170.1, 72.7, 53.2, 52.9, 23.7, 21.8, 20.3; $[\alpha]_D^{24}$ −0.6 (c 0.46, $CHCl_3$); HRMS (ESI-TOF) (m/z) [M + H]$^+$ calcd for $C_8H_{14}NO_4$ 188.0913, found 188.0917.

Fmoc-Ala-oxaPip-Gly-OtBu (16). Compound **10** (188 mg, 873 μmol) was dissolved in DMF, followed by the addition of *tert*-butyl glycinate hydrochloride (293 mg, 1.75 mmol), HCTU (542 mg, 1.31 mmol), and DIEA (623 μL, 3.49 mmol). The solution was stirred for 18 h and then concentrated. The crude material was purified over silica gel via flash chromatography (50−90% EtOAc/hexanes), yielding a yellow oil (222 mg, 77% yield): ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.99 (bs, 1H), 5.93 (m, 1H), 5.24 (dd, J = 16.0 Hz, 1.0 Hz, 1H), 5.14 (dd, J = 9.3 Hz, 1.0 Hz, 1H), 4.69 (m, 1H), 4.56 (m, 2H), 3.95−3.69 (m, 4H), 2.25 (m, 1H), 1.85 (m, 1H), 1.65 (m, 1H), 1.46 (m, 1H), 1.33 (s, 9H); ^{13}C{^{1}H} NMR (125 MHz, $CDCl_3$) δ 169.7, 168.6, 155.5, 132.0, 118.9, 82.1, 71.6, 67.3, 57.8, 42.2, 28.1, 22.9, 21.0; HRMS (ESI-TOF) (m/z) [M + Na]$^+$ calcd for $C_{15}H_{24}N_2NaO_6$ 351.1527, found 351.1534.

A portion of Alloc-oxaPip-Gly-O*t*Bu above (210 mg, 640 μmol) was dissolved in DCM. Phenylsilane (396 μL, 3.20 mmol) and $Pd(PPh_3)_4$(37.0 mg, 32.0 μmol) were added, and the solution was stirred 1 h. The reaction was concentrated and the crude material purified over silica gel via flash chromatography (0−60% EtOAc/hexanes). The purified amine was immediately dissolved in DCM and treated with Fmoc-Ala-Cl (842 mg, 2.56 mmol) and $NaHCO_3$ (430 mg, 5.12 mmol). The mixture was stirred for 18 h, after which water was added, and the organic layer separated. The aqueous layer was extracted with DCM, and the combined organic layers were dried over anhydrous Na_2SO_4. The volatiles were removed under reduced pressure, and the crude material was purified over silica gel via flash chromatography (0−60% EtOAc/hexanes), yielding a white solid (185 mg, 54% over two steps): ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.73 (m, 2H), 7.60 (m, 2H), 7.37 (m, 2H), 7.29 (m, 2H), 6.70 (bs, 1H), 5,81 (bs, 1H), 5.18 (m, 1H), 4.84 (m, 1H), 4.35 (m, 2H), 4.25−4.12 (m, 2H), 4.07−3.96 (m, 2H), 3.86 (dd, J = 14.0, 4.3 Hz, 1H), 2.39 (m, 1H), 2.03 (m, 1H), 1.81 (m, 1H), 1.60 (m, 1H), 1.45 (m, 12H); ^{13}C{^{1}H} NMR (125 MHz, $CDCl_3$) δ 172.8, 168.8, 168.4, 155.9, 143.9, 143.7, 141.2, 127.7, 127.0, 125.1, 119.9, 82.3, 73.7, 67.0. 53.7, 47.4, 47.1, 42.2, 28.0, 22.9, 20.9, 18.5; HRMS (ESI-TOF) (m/z) [M + H]$^+$ calcd for $C_{29}H_{36}N_3O_7$ 538.2548, found 538.2531.

H-Gly-Ala-oxaPip-Gly-NH$_2$ (17). Compound **16** (167 mg, 316 μmol) was dissolved in a 3:1 solution of diethylamine and MeCN and stirred for 1 h. The reaction was concentrated, and the crude material was taken up in DMF. Boc-Gly-OH (94.0 mg, 537 μmol), HATU (204 mg, 537 μmol), and DIEA (165 μL, 0.947 mmol) were added, and the reaction was stirred for 18 h. The reaction was concentrated, and the crude material was purified on silica gel via flash chromatography, resulting in a light yellow oil (91.0 mg, 61% over two steps): ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.07 (bs, 1H), 6.67 (bs, 1H), 5.40 (bs, 1H), 5.09 (s, 1H), 4.97 (m, 1H), 4.13 (m, 1H) 4.04−3.93 (m, 2H), 3.87−3.72 (m, 3H), 2.35 (m, 1H), 2.01 (m, 1H), 1.78 (m, 1H), 1.58 (m, 1H), 1.41 (m, 21H); ^{13}C{^{1}H} NMR (125 MHz, $CDCl_3$) δ 172.3, 169.3, 168.9, 168.5, 156.0, 82.4, 79.9, 73.8, 53.7, 45.9, 44.0, 42.2, 28.3, 28.0, 23.0, 20.9, 18.2; HRMS (ESI-TOF) (m/z) [M + H]$^+$ calcd for $C_{21}H_{37}N_4O_8$ 473.2606, found 473.2608.

A portion of Boc-Gly-Ala-oxaPip-Gly-O*t*Bu above (80.0 mg, 169 μmol) was dissolved in 3:1 TFA/DCM at 0 °C and allowed to warm to room temperature while stirring over 1 h. Upon completion, the reaction was concentrated, and the crude material was purified via preparative RP-HPLC (4−45% MeCN in H_2O, linear gradient with 1% TFA modifier) to provide **17** as a white solid after lyophilization (28.3 mg, 61% yield): ^{1}H NMR (500 MHz, D_2O with DSS standard) δ 5.16 (m, 1H), 4.92 (m, 1H), 4.24 (m, 1H), 4.12 (m, 1H), 3.98 (s, 2H), 3.86 (s, 2H), 2.25 (m, 1H), 2.13−1.92 (m, 2H), 1.74 (m, 1H), 1.42 (d, J = 7.2 Hz, 3H); ^{13}C{^{1}H} NMR (125 MHz, D_2O with DSS standard) δ 176.2, 174.6, 169.8, 76.5, 57.4, 49.8, 44.4, 43.2, 26.3, 23.2, 18.4; HRMS (ESI-TOF) (m/z) [M + H]$^+$ calcd for $C_{12}H_{21}N_4O_6$ 317.1456, found 317.1458.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.joc.9b02382.

^{1}H and ^{13}C NMR spectra for all new compounds, conformational assignment, and diastereopurity (PDF)

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Notes

The authors declare no competing financial interest.

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