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A FACILE SYNTHESIS OF 4-SUBSTITUTED GLUTAMATE DERIVATIVE VIA 1,3-DIPOLAR CYCLOADDITION OF DIMETHYL 2-METHYLENEGLUTARATE AND NITRONE DERIVED (-)-MENTHONE

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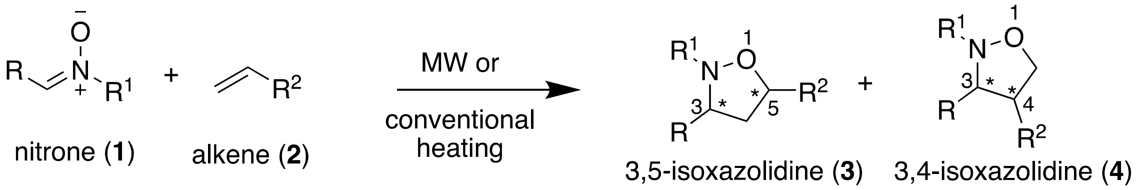
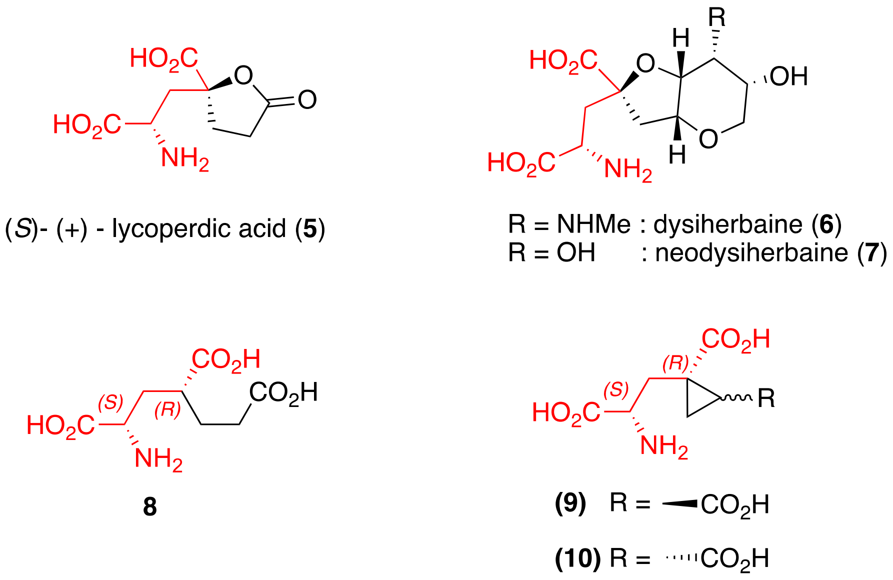
Abstract - The 1,3-dipolar cycloaddition reaction of dimethyl 2-methylene-glutarate and (-)-menthone-derived nitrone occurred with moderate selectively to produce the desired isoxazolidine which was a direct precursor to the (2*S*,4*S*)-substituted glutamate derivative in an overall yield of 20%. The possibility of preparing the unnatural amino acid, (*S*)-(+)-lycoperdic acid was studied based on its evidence within the mass spectra of the final product.

1,3-Dipolar cycloaddition of a nitrone (**1**) and an alkene (**2**) is a general method for preparing 3,5- and 3,4-isoxazolidines (**3** and **4**, respectively, in Scheme 1), each with two stereocenters.[1] The regioselectivity of the products may be governed by both steric and electronic effects of electron-rich or electron-neutral alkenes, thus favoring the 3,5-isoxazolidine (**3**) over the 3,4-isomer (**4**).[1b]



Scheme 1. General synthesis of 3,5- and 3,4-isoxazolidines

Because of the versatility of 1,3-dipolar additions, this reaction provides a simple route to a plethora of heterocyclic molecules,[2] some of which are key precursors to natural and synthetic compounds with intriguing pharmacological properties.[1a] These properties include antiviral,[3] anti-inflammatory,[4] antibacterial and antifungal activities.[5,6] *L*-Glutamate is a major neurotransmitter in the central nervous system (CNS).[7] It interacts with the ionotropic receptors (iGluRs) and metabotropic glutamate receptors (mGluRs) to coordinate the excitation of the neurons in the CNS.[8] The excitotoxicity at these receptors is involved in the onset of several neurodegenerative conditions such as stroke, epilepsy, and chronic disorders such as Parkinson’s disease and Alzheimer’s disease. Therefore, there is a continuous pursuit to identify molecules with drug-like capabilities that are possible agonist and antagonist of these receptors.[7-9] Several natural and synthetic small molecules possess this *L*-glutamate scaffold. These include the natural products (*S*)-(+)-lycoperdic acid (**5**),[10] dysiherbaine (**6**)[11] and neodysiherbaine (**7**),[12] and the glutamate analogs **8** – **10**,[9] which are agonists of selected mGluRs. (Figure 1)



(*S*)- (+) - lycoperdic acid (**5**)

R = NHMe : dysiherbaine (**6**)
R = OH : neodysiherbaine (**7**)

8

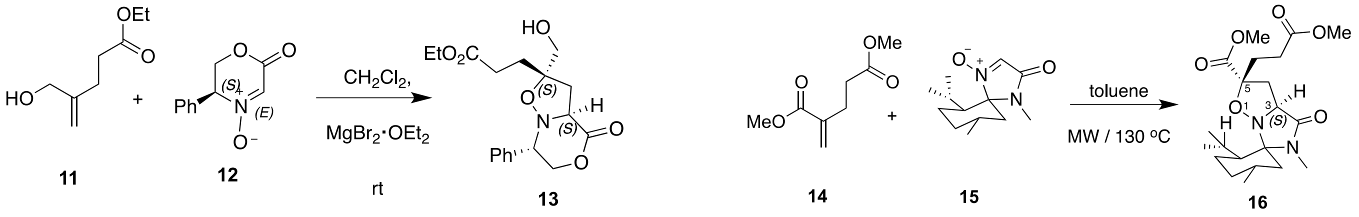
(9) R = CO_2H

(10) R = CO_2H

Figure 1. Glutamate scaffold in natural and synthetic compounds

Because of the biological significance of glutamate derivatives, we have embarked upon a project to rapidly prepare *L*-glutamate derivatives using microwave technology. This will enhance the availability of these compounds for probing the glutamate receptors, and to further comprehend and hopefully eliminate known neurological disorders such as Parkinson’s and Alzheimer’s diseases

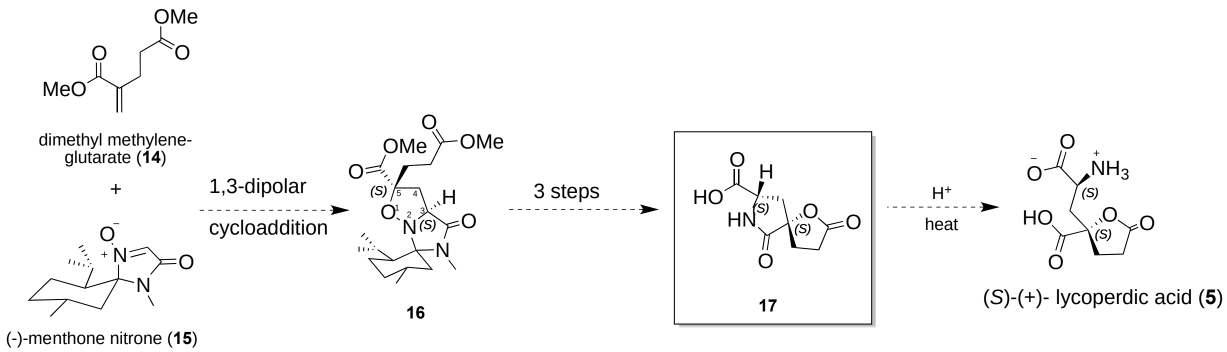
This work reports the facile synthesis of two glutamate derivatives and a synthetic study on lycoperdic acid **5**, an unnatural amino acid that was first isolated by Banga and co-workers in 1978.[10a] Although several syntheses of lycoperdic acid **5** have been reported in the literature,[10] the report by Tamura and coworkers[10e] was of interest to us because of its similarity to our study. The key step in their work involved the 1,3-dipolar cycloaddition of chiral nitrone **12** and allylic alcohol **11** in the presence of $MgBr_2 \cdot OEt_2$. This provided the isoxazolidine **13** in 94% yield with a very high selectivity of 91:9 for the desired (*S*)-stereoselectivity at C-2 and C-4 in 72 h (Scheme 2).



Scheme 2. A comparison of Tamura et al.'s 1,3-dipolar cyclization (**11** + **12** → **13**)[10e] with our analogous approach in this study (**14** + **15** → **16**)

We desired to utilize Tamura et al.'s cycloaddition strategy for the synthesis of **16** *via* the 1,3-dipolar cyclization of dimethyl 2-methyleneglutarate **14**[13] and chiral nitrone **15**[14] (Scheme 2). Furthermore, previous work by Altenbach et al. revealed that under conventional heating, nitrone **15** can effectively control the stereochemistry of the 1,3-dipolar cyclization reaction by directing the alkene selectively to the less hindered face of the molecule via an *exo*-type approach.[14a] However, a disadvantage of conventional heating (i.e., Altenbach's and Tamura's conditions) is the extended reaction times of three hours to several days.[10e,14] To circumvent this, microwave (MW) heating has been shown to provide faster reactions and may simultaneously improve the selectivity in these types of dipolar cyclization reactions.[1b] MW heating has the added benefit of increasing product yields with fewer by-products.[1c,9] The use of a MW reactor also enables the possibility of solvent-free reactions or using water as a solvent (versus an organic solvent), thus improving the environmental friendliness of the MW method over conventional heating.

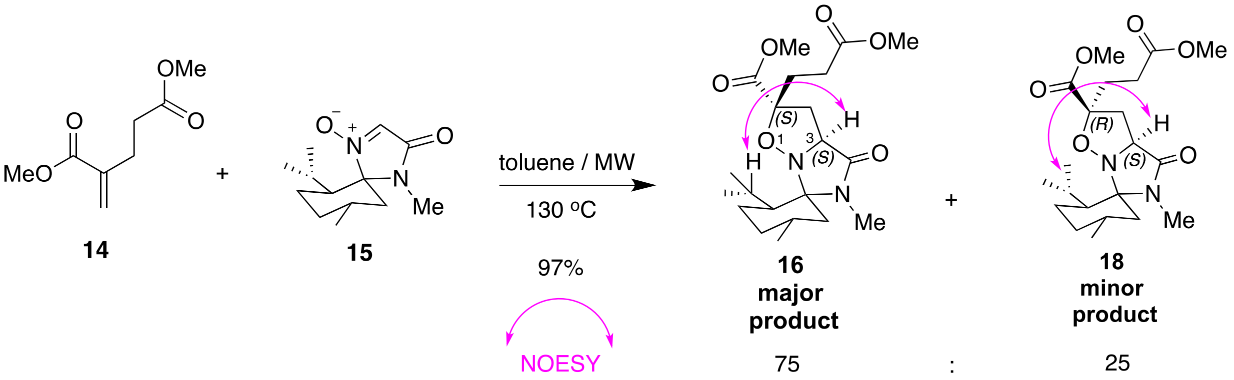
We have thus adapted Tamura et al.'s and Altenbach's cycloaddition strategies for the synthesis of glutamate derivative **17** *via* microwave-assisted 1,3-dipolar cyclization of **14** and **15**. We also studied the possibility of preparing the natural product, (*S*)-(+)-lycoperdic acid (**5**) from **17** under acidic hydrolysis conditions (Scheme 3). The details of this study are described below.



Scheme 3. Retrosynthetic approach to lycoperdic acid (**5**) via the glutamic acid derivative **17**

Dimethyl 2-methyleneglutarate **14** and the nitrone **15** were prepared from readily available starting materials according to literature procedures. Initially, **14** and **15** were reacted under MW conditions at

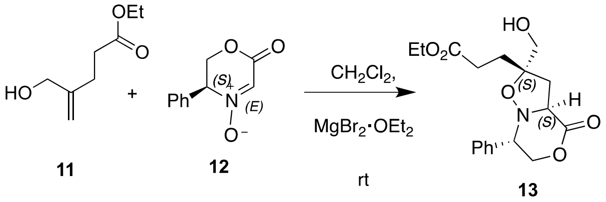
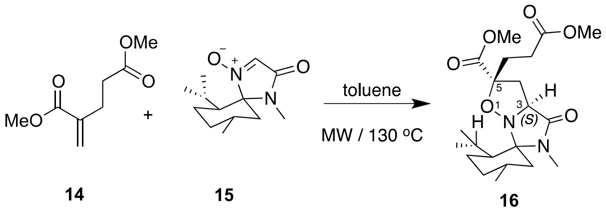
130 °C in toluene. The regioselectivity favored the desired isoxazolidine, **16**, and its diastereoisomer **18** in excellent yield (97%) and with a diastereomeric ratio (dr) of 75:25 (Scheme 4). The regioselectivity was as expected with the methylene carbon of **14** attacking the electrophilic carbon of the nitrone **15**.[14b] The observed stereoselectivity resulted from alkene **14** adding to the less hindered face of **15** (i.e., away from the isopropyl group) in an *exo*-type approach.[14b,15]



Scheme 4. 1,3-Dipolar cyclization reaction of dimethyl 2-methyleneglutarate **14** and (-)-nitrone **15**

In comparison to Tamura and co-workers' results with conventional heating,[10e] we increased the 1,3-dipolar cycloaddition yield to 97% in a much shorter time of 1 h under microwave conditions, with moderate stereoselectivity (75:25) using the alkene **14**[13] and the chiral auxiliary **15**[14] (Table 1). This result was promising for our study as both diastereomers were readily separated using flash column chromatography.

Table 1. Comparison of the results from the 1,3-dipolar cycloaddition step in the synthesis of 4-substituted glutamate derivatives by Tamura *et al.*[10e] and our work

Reference	Key step	% Yield	Selectivity	Reaction time (h)
10e		94	91:9	72
This work		97	75:25	1

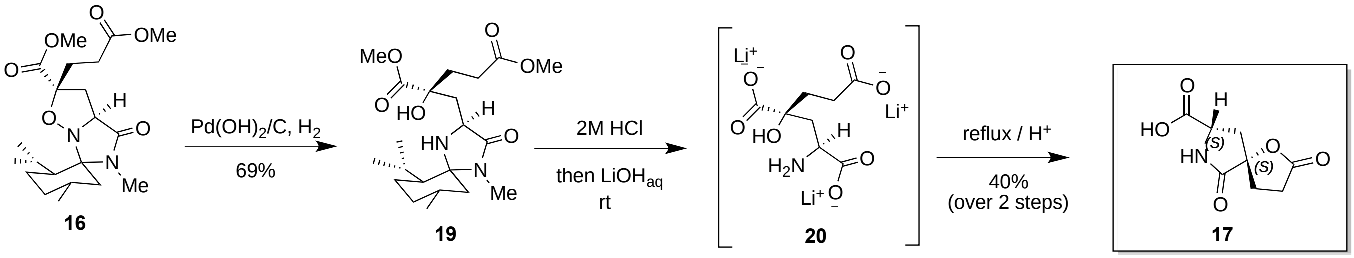
Diastereomers **16** and **18** were therefore separated using flash column chromatography. Careful analysis of their ^{1}H NMR, COSY, and NOESY spectra led to partial characterization of these compounds. In the NOESY spectra of **16**, the methine proton 3-H that resonated at 3.89 ppm showed correlation to the isopropyl methine H at 1.35 ppm. In **18**, a similar relationship was observed for the 3-H proton at 3.87 ppm and the isopropyl methyl protons at 1.43 ppm. These NOESY correlations established the *S* configuration at C-3 in both isomers. Subsequent reductions and/or hydrolyses under acidic and/or basic conditions provided derivatives whose NMR spectra further helped to locate the stereochemistry at the stereocenters in **16.**

We then undertook a brief study of the effect of MW heating compared to conventional heating on this reaction (Table 2). Most impressively, MW heating reduced the reaction time to 1.0 h as compared to 21.5 h with conventional heating. Additionally, the overall product yield improves to 97% under MW heating at 130 °C as compared to 93% with conventional heating. However, diastereoselectivity is better under conventional heating (83:17 dr) versus MW heating at 130 °C (75:25 dr). We also noted that greater amounts of the minor diastereomer, **18**, were formed at elevated temperature (i.e., MW heating at 150 °C), thus supporting **18** as the thermodynamic product. We plan future studies of this phenomenon.

Table 2. Effect on the 1,3-dipolar cycloaddition of dimethyl 2-methyleneglutarate (**14**) and (-)-menthone nitrone (**15**) on product selectivity under conventional and MW conditions

Condition	T (°C)	% Yield	Isoxazolidine ratio 16 : 18	Reaction time (h)
reflux	110	93	83:17	21.5
MW	130	97	75:25	1.0
MW	150	93	67:33	1.0

To progress on our synthesis of glutamate derivative (**17**), the major diastereomer, isoxazolidine **16**, was then subjected to a variety of reductive conditions to give amino alcohol **19** (Scheme 5). As shown in Table 3, the best reduction was obtained when Pd $(OH)_2/C$ was used as a catalyst, giving **19** in 69% yield (Entry 3).



Scheme 5. Synthesis of glutamate derivative **17** from isoxazolidine **16**

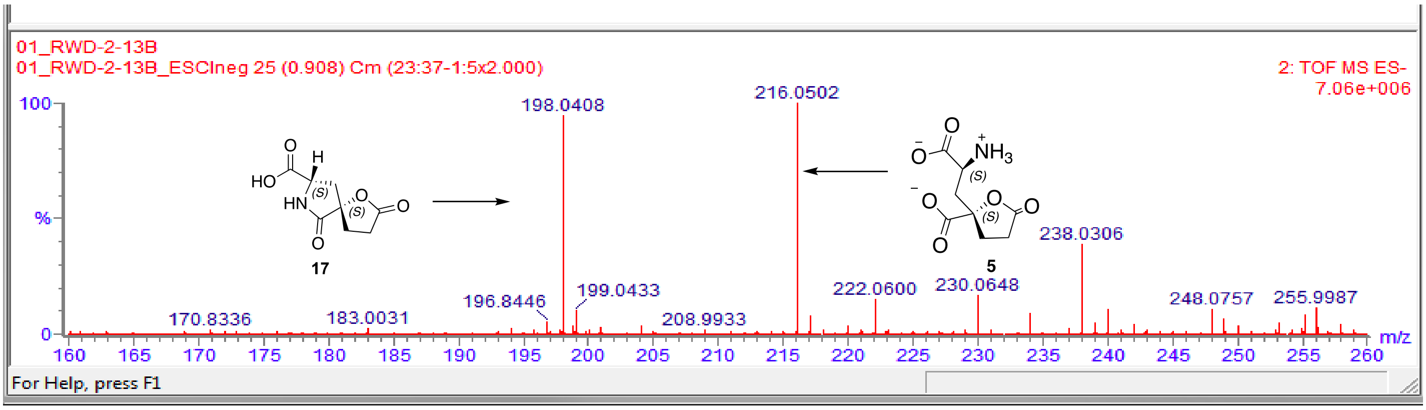
Table 3. Reductive cleavage of isoxazolidine **16** to amino alcohol **19**

Entry	Reaction conditions	% Yield[a]
1	polymethylsiloxane; $Pd(OH)_2/C$[16]	9
2	H_2; HCO_2NH_4[17]	33
3	$Pd(OH)_2/C$, H_2	69 (33% conversion)

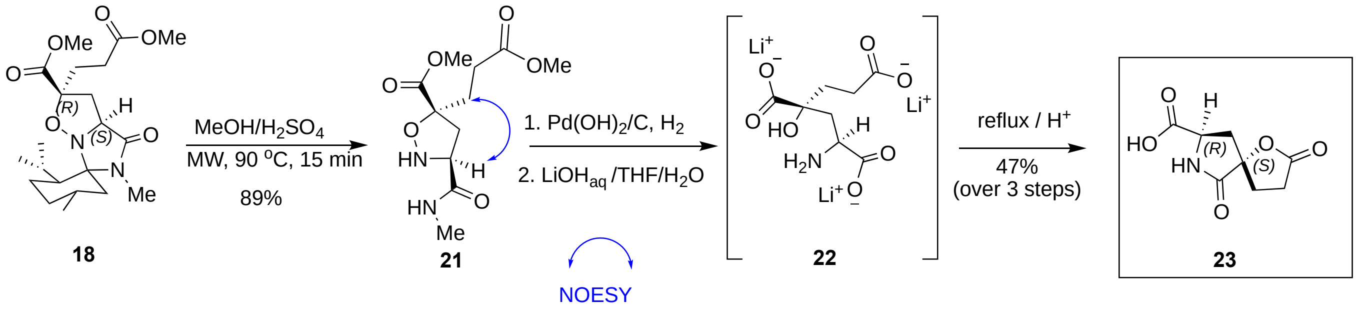
[a] Yield based on unreacted **16** recovered from the reaction mixture

Acidic cleavage of the chiral auxiliary of **19** and subsequent amide hydrolysis with aqueous LiOH provided the globally hydrolyzed product **20**. Crude **20** was then refluxed with 6 M HCl (aq) to give the glutamate derivative **17** after ion exchange chromatography with 2 N acetic acid solution in 28% yield from **16**. Compound **17** was characterized from its ^1H and ^{13}C NMR spectra, and mass spectral data. (TOF MS (m/z = 198.0408 $[M-H]^-$) (Figure 2). Noteworthy is the signal at m/z = 216.0508 $[M-H]^-$ which indicated the presence of the natural product lycoperdic acid (**5**) in the transformation of **19** to **17**. Further acid hydrolysis of **17** with 6 M HCl did not improve the production of **5**. Hence an alternate route to **5** is being considered for future examination starting with isoxazolidine **16**.

Figure 2. TOF ES^- mass spectrum of the reaction mixture of **17**



We also proceeded with the synthesis of the (2*S*,4*R*)-diastereomer of **17**, which is the glutamate derivative (**23**), from minor isoxazolidine **18** (Scheme 5) in a slightly different way from the previous route (i.e., **16** → **19** → **20** → **17**). We believed the conversion of isoxazolidine **16** to amino alcohol **19** was very low due to the strain in **16**, which restricted the reduction of the N-O bond. To increase the yield of the analogous conversion of **18** to **21**, the acid hydrolysis of **18**'s menthone auxiliary group was conducted under MW conditions in methanol, rendering isoxazolidine **21** in 89% yield. The methoxy groups of the ester and the *N*-methylamide group were unaffected by these reaction conditions, as evident from the ^{1}H NMR spectra: singlets at 3.83 and 3.68 ppm, and the doublet at 2.85 ppm (J = 6.7 Hz), respectively. Two-dimensional NOESY on **21** was used to assign the methine proton at C-3 and the indicated methylene protons. This NOESY data ultimately confirmed the 2*S* and 4*R* configuration of **23** (Scheme 6).



Scheme 6. Synthesis of glutamate derivative **23** from isoxazolidine **18**

To convert isoxazolidine **21** to the glutamate derivative **23**, **21** was first hydrogenated with Pd(OH)$_2$/C as the catalyst to effect N-O cleavage. Then, the amide and ester were hydrolyzed under basic conditions (LiOH·H$_2$O) to give lithium carboxylate salt **22**. Treatment of crude **22** with refluxing 6 M HCl resulted in formation of the glutamate derivative **23**. Purification of the reaction mixture via ion exchange chromatography in 2 N acetic acid solution provided pure **23** in 47% yield after three steps (i.e., **21** → **23**).

In conclusion, the syntheses of the glutamate derivatives **17** and **23** were prepared with moderate stereoselectivity via the microwave-assisted 1,3-dipolar cycloaddition of dimethyl 2-methyleneglutarate **14** and the (-)-menthone nitrone **15**. Global reductive cleavage of the N-O bond of each diastereomeric isoxazolidine followed by base hydrolysis of the amide and ester, and acid-catalyzed lactonization gave **17** and **23** in 20% and 10% overall yield. Further acid hydrolysis of **17** over extended period was unsuccessful in producing isolable amount of the lycoperdic acid (**5**). Hence, a revised synthesis of **5** via the isoxazolidine **16** is presently occurring in our lab. Biological testing of **17** and **23** against the ionotropic glutamate receptor, 2-carboxy-3-carboxymethyl-4-isopropenylpyrrolidine (kainic acid, KA)[11] are also planned.

EXPERIMENTAL

General experimental. Melting points were obtained on a Laboratory Devices MEL-TEMP II melting point apparatus and are uncorrected. A Perkin-Elmer Spectrum 100 FTIR Spectrometer was used to obtain IR spectra. A Bruker Avance 300 MHz FT-NMR spectrometer, using TMS or solvent peaks as reference, was used to obtain ^{1}H and ^{13}C NMR spectra in deuterated solvents. Compounds **16**, **17**, **18**, **19**, **21** and **23** were characterized by IR, ^{1}H NMR, ^{13}C NMR, and HRMS spectral data. Thin layer chromatography (TLC) was conducted on pre-coated silica gel plates that were visualized under UV light (254 nm) and developed with potassium permanganate stain solution or, in the case of amines, EtOH in ninhydrin solution. Microwave (MW) reactions were carried out with a CEM Discover SP microwave reactor. The ratio of diastereomeric isoxazolidines **16** and **18** was determined by comparing the ^{1}H NMR integrals of the *N*-methyl protons in the crude product mixture from the MW-assisted 1,3-dipolar cycloaddition.

Dimethyl 2-methyleneglutarate (14):[13] Methyl acrylate (21.5 g, 250 mmol) was cooled to -10 °C and tributylphosphine (5.1 g, 25 mmol) was added under an N_2 atmosphere in a 250-mL round-bottom flask. The reaction was allowed to warm to room temperature with stirring over 1.5 h. After 1.5 h, the reaction mixture was concentrated by rotary evaporation to give the crude product. Flash column chromatography of the crude product with 10% EtOAc / cyclohexane eluent gave **14** (11.8 g, 56%) as an oil. IR (neat, ν_{max}, cm^{-1}): 2996, 2953, 2846, 1718, 1631, 1437; ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.18 (s, 1H), 5.59 (s, 1H), 3.75 (s, 3H), 3.66 (s, 3H), 2.63 (t, *J* = 7.2 Hz, 2H), 2.51 (t, *J* = 7.2 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 173.2, 167.2, 138.8, 126.1, 52.0, 51.7, 33.0, 27.4.

Procedure for the synthesis of isoxazolidines 16 and 18: Nitrone **15**[14] (0.231 g, 0.97 mmol) was added to a 10-mL MW reaction tube with alkene **14** (0.505 g, 2.94 mmol, 3 equivalents) in 1.5 mL of toluene. The reaction mixture was heated in a microwave reactor at 130 °C and 200 W for 1 h, and then analyzed via TLC. The reaction mixture was concentrated by removing the solvent via rotary evaporation to give crude **16**, **18** and unreacted **14** as a yellow oil (0.87 g). ^{1}H NMR of the crude reaction mixture prior to purification established the diastereomeric ratio (dr) of **16**:**18** as 75:25. The residue was purified by flash column chromatography (silica gel, 5-20% EtOAc/CH_2Cl_2) to afford compounds **18** (0.049 g), mixture **16** and **18** (0.265 g), and **16** (0.0744 g), as a pale yellow oils (0.388 g, 97%)

Methyl (1*S*,2*S*,2'*S*,3a'*S*,5*R*)-2-isopropyl-2'-(3-methoxy-3-oxopropyl)-5,5'-dimethyl-4'-oxotetrahydro-2'*H*-spiro[cyclohexane-1,6'-imidazo[1,5-*b*]isoxazole]-2'-carboxylate (16): $[\alpha]_D^{20}$ +81.2 (c 3.3, CH_2Cl_2); IR (neat, ν_{max}, cm^{-1}): 2952, 2932, 2870, 1737, 1697, 1436; ^1H NMR (300 MHz, $CDCl_3$): δ 3.89 (dd, *J* =2.0, 8.1 Hz, 1H), 3.68 (s, 3 H), 3.65 (s, 3H), 3.03 (dd, *J* = 2.0, 13.0 Hz, 1H), 2.71 (s, 3H, N-CH_3), 2.52 (m, 1H), 2.49 - 2.12 (m, 5H), 1.93 (m, 1H), 1.83 (m, 1H), 1.71 (dd, *J* = 2.4, 12.0 Hz, 1H), 1.62 (m, 1H), 1.28 - 1.48 (m, 2H), 1.18 (t, *J* = 12.3 Hz, 1H), 0.94 (d, *J* = 6.5 Hz, 3H), 0.90 (m, 1H), 0.84 (d, *J* = 6.8 Hz, 3H), 0.79 (d, *J* = 6.7 Hz, 3H); ^{13}C -NMR (75 MHz, $CDCl_3$): δ 173.3, 172.3, 172.0, 89.2, 82.5, 65.4, 52.7, 51.9, 48.4, 41.3, 40.8, 35.0, 32.4, 29.4, 29.3, 26.1(N-$\underline{C}H_3$), 24.4, 24.3, 22.7, 22.5, 18.8; HRMS(ESI): *m/z* calc for $C_{21}H_{35}N_2O_6$ $[M + H]^+$ 411.2495, found 411.2497.

Methyl (1*S*,2*S*,2'*S*,3a'*S*,5*R*)-2-isopropyl-2'-(3-methoxy-3-oxopropyl)-5,5'-dimethyl-4'-oxotetrahydro-2'*H*-spiro[cyclohexane-1,6'-imidazo[1,5-*b*]isoxazole]-2'-carboxylate (18): IR (neat, ν_{max}, cm^{-1}): 2952, 2928, 2870, 1738, 1693, 1436, 729; ^1H NMR (300 MHz, $CDCl_3$): δ 3.86 (dd, *J* = 3.8, 10.0 Hz, 1H), 3.74 (s, 3 H), 3.66 (s, 3 H), 3.11 (dd, *J* = 10.0, 13.8 Hz, 1H), 2.73 (s, 3H , N-CH_3), 2.03 - 2.38 (m, 7H), 1.82 (m, 1H), 1.51 - 1.69 (m, 2H), 1.43 (sextet, *J* = 6.8 Hz, 1H), 1.36, (dd, *J* = 3.5, 12.1 Hz, 1H), 1.21 (t, *J* = 12.1 Hz, 1H), 0.93 (d, *J* = 6.5 Hz, 3H), 0.90 (m, 1H), 0.84 (d, *J* = 6.9 Hz, 3H), 0.73 (d, *J* = 6.7 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 173.7, 173.3, 173.0, 87.8, 82.6, 65.0, 52.6, 52.0, 48.2, 41.3, 38.7, 35.0, 30.2, 29.2, 28.9, 26.1(N-$\underline{C}H_3$), 24.5, 24.3, 22.7, 22.5, 18.8; HRMS(ESI): *m/z* calc for $C_{21}H_{35}N_2O_6$ $[M + H]^+$ 411.2495, found 411.2495.

(5*S*,8*S*)-2,6-Dioxo-1-oxa-7-azaspiro[4.4]nonane-8-carboxylic acid (17): A solution of isoxazolidine **16** (155 mg, 0.38 mmol) and 20% of palladium hydroxide on carbon (49 mg) in MeOH (3 mL) in a 50-mL round-bottom flask was reacted under H_2 gas (balloon) in a 50 mL round bottom flask for 48 h. The reaction solution was filtered through Celite®, washed with 20 mL of MeOH, and concentrated via rotary evaporation. The crude product was purified by column chromatography on silica gel (10% EtOAc/ $CHCl_3$ eluent) to give amino alcohol **19** as a colorless oil (52 mg, 69% yield based on recovered **16**). IR (neat, ν_{max}, cm^{-1}): 3339, 2952, 2927, 2870, 1789, 1738, 1435; ^1H NMR (300 MHz, $CDCl_3$) δ: 3.75 (s, 3H), 3.67 (apparent t, *J* = 5.6 Hz, 1H), 3.65 (s, 3H), 2.72 (s, 3H), 2.52 (m, 1H), 1.97-2.30 (m, 5H), 1.85 - 1.23 (m, 10 H), 0.94 - 0.80 (m, 10H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 176.5, 174.3, 173.9, 81.6, 75.6, 55.7, 52.8, 51.9, 48.3, 46.8, 34.9, 34.6, 29.1, 28.7, 25.5, 24.8, 24.1, 22.4, 22.3, 18.6; HRMS(ESI): *m/z* calcd for $C_{21}H_{37}N_2O_6$ $[M + H]^+$ 413.2652, found 413.2653. Then, to a 25-mL round-bottom flask was added amino alcohol **19** (15 mg, 0.036 mmol), EtOH (3.0 mL), and 6 M HCl (0.5 mL). This mixture was refluxed (80 °C) for 18 h. The solvent was then removed under vacuum. The resulting crude residue was mixed with 3 mL of water/THF (2:1) and to this mixture was added lithium hydroxide monohydrate

(8.0 mg, 0.19 mmol). This reaction mixture was stirred at room temperature for 2 h. After evaporating the reaction solvent under vacuum, the resulting crude product **20** was dissolved in 2 mL of 6 M HCl and refluxed for 2 h. The water solvent was removed under vacuum and the crude residue was purified on a reverse-phase column (Dowex 50W X8, 200-400 mesh, hydrogen form) with 2 N acetic acid as the eluent. The 4-substituted glutamate derivative **17** (3.9 mg, 46%) was produced as a white amorphous solid. $[\alpha]_D^{20}$ + 43.9 (c 0.23, H_2O); IR (neat, ν_{max}, cm^{-1}): 3372, 1764, 1706, 1634, 1415; ^1H NMR (300 MHz, D_2O): δ 4.38 (dd, *J* = 3.7, 9.2 Hz, 1H), 2.90 (dd, *J* = 9.7, 14.2 Hz, 1H), 2.86 - 2.74 (m, 2H), 2.57 (m, 2H), 2.30 - 2.15 (m, 1H); ^{13}C NMR (75 MHz, D_2O): δ 179.9, 176.0, 175.6, 86.2, 52.5, 36.4, 30.0, 28.2; HRMS(ESI): *m/z* calcd for $C_8H_8NO_5$ $[M - H]^-$ 198.0402, found 198.0408.

Methyl (3*S*,5*R*)-5-(3-methoxy-3-oxopropyl)-3-(methylcarbamoyl)isoxazolidine-5-carboxylate (21): A solution of isoxazolidine **18** (184 mg, 0.448 mmol) in anhydrous MeOH (2 mL) was placed in a 10-mL MW reaction tube. To it was added concentrated sulfuric acid (0.10 mL) and concentrated (glacial) acetic acid (1 mL). The reaction solution was then heated in a MW reactor at 90 °C and 200 W for 15 min. After cooling, 10 mL of a saturated aqueous $NaHCO_3$ solution was added to the reaction mixture. The mixture was subsequently extracted with CH_2Cl_2 (3 x 15 mL) and the combined organic layers were washed with water (1 x 10 mL), brine (1 x 10 mL), and dried over anhydrous Na_2SO_4. Rotary evaporation of the solvent organic layer provided a brown oil. Flash column chromatography (10-80% EtOAc/CH_2Cl_2) of the crude residue provided **21** as a yellow oil (39.4 mg, 89% based on recovered **18**). IR (neat, ν_{max}, cm^{-1}): 3388, 3256, 2955, 2926, 2854, 1733, 1662, 1536, 1438; ^1H NMR (300 MHz, $CDCl_3$): δ 7.31 (brd s, 1H, -N$\underline{H}$-CH_3), 6.49 (d, *J* = 5.7 Hz, 1H, O-N$\underline{H}$), 3.98 (quintet, *J* = 4.5Hz, 1H), 3.77 (s, 3H), 3.65 (s, 3H), 2.84 (d, *J* = 5.0 Hz, 3H, C$\underline{H_3}$NH-), 2.69 (dd, *J* = 7.1, 14.1 Hz, 1H), 2.65 (dd, *J* = 3.5, 14.1 Hz, 1H), 2.45 - 2.02 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 173.7, 173.0, 171.7, 87.6, 62.3, 52.9, 52.0, 42.8, 30.2, 29.1, 26.2; HRMS(ESI): *m/z* calcd for $C_{11}H_{19}N_2O_6$ $[M + H]^+$ 275.1243, found 275.1235.

(5*R*,8*S*)-2,6-Dioxo-1-oxa-7-azaspiro[4.4]nonane-8-carboxylic acid (23): A solution of isoxazolidine **21** (38.0 mg, 0.138 mmol) and 20% of palladium hydroxide on carbon (40 mg) in MeOH (3 mL) was reacted in a 50-mL round-bottom flask under H_2 gas (balloon) for 48 h. The reaction solution was filtered through Celite®, washed with 20 mL of MeOH, and concentrated via rotary evaporation. The resulting crude residue was mixed with 3 mL of water/THF (2:1) and to this mixture was added lithium hydroxide monohydrate (32.0 mg, 0.76 mmol). This reaction mixture was stirred at room temperature for 2 h. After evaporating the reaction solvent under vacuum, the resulting salt **22** was dissolved in 2 mL of 6 M HCl (aq) and refluxed for 18 h. The water solvent was removed under vacuum and the crude residue was purified on a reverse-phase column (Dowex 50W X8, 200-400 mesh, hydrogen form) with 2 N acetic acid as eluent. After removal of the water under vacuum, the crude residue was purified on a reverse phase column (Dowex 50W X8, 200-400 mesh, hydrogen form) with 2 N acetic acid as the eluent. The 4-substituted glutamate derivative **23** was produced as a white amorphous solid (13.0 mg, 47%). ^{1}H NMR (300 MHz, D_2O): δ 4.41 (dd, J = 5.5, 8.4 Hz, 1H), 2.95 - 2.70 (m, 3H), 2.64 - 2.47 (m, 2H), 2.44 - 2.31 (m, 1H); ^{13}C NMR (75 MHz, D_2O): δ 179.8, 175.7, 175.1, 86.2, 52.2, 36.4, 29.0, 28.2; HRMS(ESI): *m/z* calcd for $C_8H_8NO_5$ $[M - H]^-$ 198.0402, found 198.0406.

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Supporting Information: Full experimental detail and characterization data can be found via the ‘Supplementary Content’ section of this article’s webpage.

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