Organic Syntheses

Preparation of *(S)*-*N*-Boc-5-oxaproline

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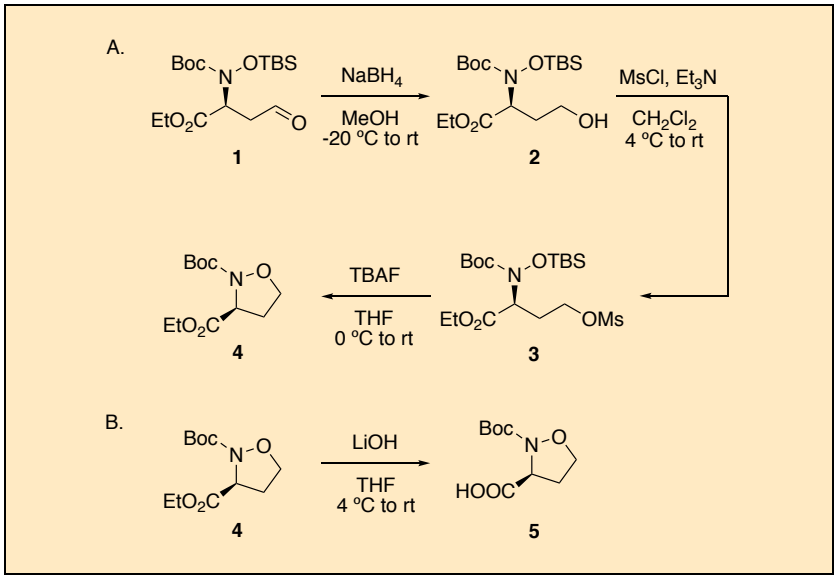

Procedure (Note 1)

A. *2-(tert-Butyl) 3-ethyl (S)-isoxazolidine-2,3-dicarboxylate* ***(4)***. The aldehyde **1** (15.0 g, 39.9 mmol, 1.0 equiv, 94% ee) (Note 2) is dissolved in MeOH (200 mL, concentration of substrate is 0.20 M) (Note 3) in a 500-mL, three-necked, round-bottomed flask equipped with a 4-cm Teflon-coated magnetic stir-bar, a plastic stopper, a low-temperature thermometer and a rubber septum through which a positive nitrogen atmosphere is ensured

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(Note 3) (Figure 1) . The reaction mixture is cooled to –20 ºC using an CH_3CN-dry ice bath. Sodium borohydride (3.02 g, 79.8 mmol, 2.0 equiv) is added in ten portions (~300 mg every three min) via the neck with the

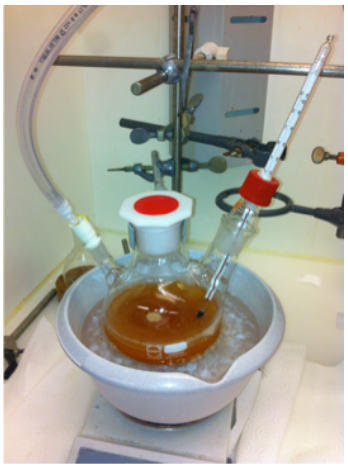


Figure 1. Glassware assembly for the reduction step (picture obtained from submitters)

plastic stopper, and the internal temperature is maintained at –20 ºC. After complete addition, the reaction is stirred for 45 min in the CH_3CN-dry ice bath at –20 ºC, after which the reaction is allowed to warm up to 0 ºC. The reaction is monitored by TLC in 25% EtOAc in hexanes using ninhydrin to stain (Note 4). After stirring for 35 min at 0 ºC, TLC analysis shows disappearance of starting material. To the completed reaction, a mixture of ice-water (170 mL) is added to the solution with vigorous stirring and the solution is stirred for 10 min at 0 ºC (Note 5). To this mixture EtOAc (900 mL) and H_2O (50 mL) are added and the resultant mixture is poured into a 2-L separatory funnel. Ethyl acetate (50 mL) and H_2O (50 mL) are used to rinse the flask. The aqueous layer is extracted with EtOAc (3 x 150 mL). The combined organic layers are washed with saturated aqueous NH_4Cl (100 mL) and saturated aqueous NaCl (100 mL). The organic layer is dried over Na_2SO_4 (20 g) and filtered by suction using a fritted funnel (9 cm diameter, medium porosity). Additional EtOAc (50 mL) is used to wash the Na_2SO_4 and the filtrate is concentrated by rotary evaporation into a 2-L round-bottomed flask (40 °C bath, 140–30 mmHg). The resulting pale yellow oil, containing ethyl *N*-(*tert*-butoxycarbonyl)-*N*-((*tert*-

butyldimethylsilyl)oxy)-L-homoserinate (i.e., **2**) is transferred to a 500-mL, single-necked round-bottomed flask using CH_2Cl_2, which is then evaporated (40 °C bath, 440–30 mmHg) on a rotary evaporator. The flask is equipped with a 4-cm oval Teflon-coated magnetic stir-bar, and the viscous oil stirred while being dried on the vacuum pump (0.15 mmHg, 24 °C) for 5 h to obtain a pale yellow oil **2** (14.7 g). The material is used without further purification (Notes 6 and 7).

The oil **2** (14.7 g, 38.9 mmol, 1.0 equiv) is diluted with CH_2Cl_2 (175 mL) and transferred, using a long-stemmed plastic funnel, into a 500-mL, three-necked, round-bottomed flask equipped with a 4-cm Teflon-coated magnetic stir-bar, a 125-mL addition funnel, a thermometer fitted with a glass adaptor, and a rubber septum through which an active nitrogen atmosphere is ensured (Figure 2). The flask from which **2** is transferred is

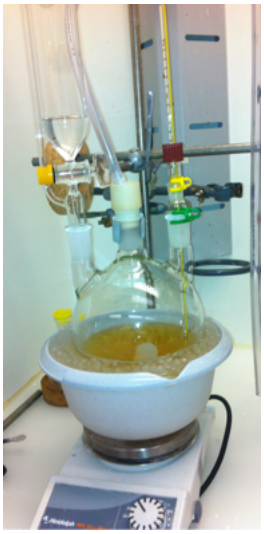


Figure 2. Glassware assembly for the mesylation step (picture obtained from submitters)

washed with CH_2Cl_2 (10 mL) to ensure that no product is left. The receiving flask is cooled to 4 °C using an ice-water bath. The addition funnel is charged with Et_3N (16.6 mL, 119 mmol, 3.0 equiv, via 20-mL disposable syringe), which is then added dropwise over 15 min maintaining the internal temperature to 3–4 °C. The addition funnel is washed with CH_2Cl_2 (5 mL) to ensure that no reagents are left. The same addition funnel is next

charged with methanesulfonyl chloride (7.40 mL, 95.6 mmol, 2.40 equiv, via 10-mL disposable syringe) which is then added dropwise over approximately 20 min. The internal temperature is maintained at 4 ºC through the entire course of the addition. The addition funnel is washed using CH_2Cl_2 (5 mL) to ensure that no reagents are left (final concentration of substrate is 0.2 M). The addition funnel is removed and the flask is equipped with a glass stopper. The reaction mixture is stirred for 15 min at 4 ºC after which the ice-water bath is removed and the reaction is stirred at 24 ºC for 1 h. The reaction is monitored by TLC in 40% EtOAc in hexanes using ninhydrin stain (Note 8). After stirring for 1 h at 24 ºC, TLC analysis shows disappearance of starting material. To the completed reaction, saturated aqueous NH_4Cl (150 mL) is added and the mixture is poured into a 2-L separatory funnel. The flask is washed with CH_2Cl_2 (10 mL) to ensure that no reagents are left. The aqueous layer is separated and extracted with CH_2Cl_2 (4 x 150 mL). The combined organic layers are washed sequentially with saturated aqueous NH_4Cl (200 mL), saturated aqueous $NaHCO_3$ (200 mL) and saturated aqueous NaCl (200 mL). The organic layer is dried over Na_2SO_4 (20 g) and filtered by suction using a fritted funnel (9 cm diameter, medium porosity). Dichloromethane (25 mL) is used to wash the Na_2SO_4 and the filtrate is concentrated by rotary evaporation into a 2-L round-bottomed flask (40 °C bath, 440–30 mmHg). The resulting dark-orange oil, containing ethyl *N*-(*tert*-butoxycarbonyl)-*N*-((*tert*-butyldimethylsilyl)oxy)-*O*-(methylsulfonyl)-L-homoserinate (i.e., **3**) is transferred to a 1-L round-bottomed flask using CH_2Cl_2, which is then evaporated (40 °C bath, 440–30 mmHg) on a rotary evaporator. The residue is transferred to a pre-weighed 250-mL single-necked round-bottomed flask equipped with pre-weighed 4-cm oval Teflon-coated magnetic stir-bar, and dried while stirring on the vacuum pump (0.15 mmHg, 24 °C) for 6 h to afford a dark-orange oil **3** that is used in the next step with no further purification (17.7 g) (Note 9).

The compound **3** (17.7 g, 38.8 mmol, 1.0 equiv) is diluted with THF (750 mL) and transferred, using a long-stemmed plastic funnel, into a 1-L, three-necked, round-bottomed flask equipped with a 4-cm Teflon-coated magnetic stir-bar, a 125-mL addition funnel, a thermometer fitted with a glass adaptor, and a rubber septum through which an active nitrogen atmosphere is ensured. THF (15 mL) is used to rinse the flask and the remaining solution transferred into the reaction flask using a 10-mL pipette. The flask is cooled down to 4 ºC using an ice-water bath (Figure 3). The addition funnel is charged with tetrabutylammonium fluoride (1 M in THF,

58.0 mL, 58.0 mmol, 1.50 equiv, via a 100-mL graduated cylinder), which is then added to the reaction flask dropwise over 90 min, maintaining the internal temperature to 3–4 ºC. Tetrahydrofuran (10 mL) is used to ensure that no reagents are left on the side of the addition funnel (final concentration of substrate 0.05 M). The addition funnel and the

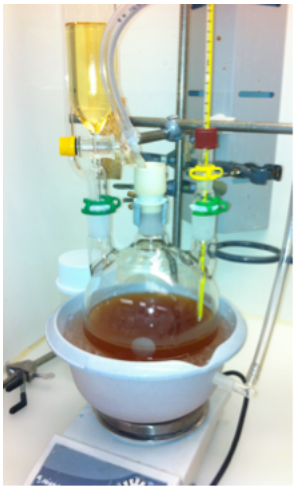


Figure 3. Glassware assembly for the cyclization step (picture obtained from submitters)

thermometer are removed and the flask is equipped with a glass stopper and a rubber septum. The reaction mixture is stirred for 1 h at 4 ºC. The reaction is monitored by TLC in 40% EtOAc in hexanes using ninhydrin to stain (Notes 10 and 11). Upon completion of the reaction (as noted by disappearance of starting material (Notes 10 and 11), saturated aqueous $NaHCO_3$ (120 mL) is added to the reaction and stirred for 10 min. The resultant biphasic mixture is diluted with Et_2O (300 mL) and the mixture poured into a 2-L separatory funnel. The aqueous layer is separated and washed with Et_2O (3 x 150 mL). The combined organic layers are washed sequentially with saturated aqueous $NaHCO_3$ (150 mL) and saturated aqueous NaCl (150 mL). The organic layer is dried over Na_2SO_4 (30 g) and filtered by suction using a fritted funnel (9 cm diameter, medium porosity). Diethyl ether (200 mL) is used to wash the Na_2SO_4 and the filtrate is concentrated by rotary evaporation (40 °C bath, 500–30 mmHg). The resulting brown oil, containing (*S*)-2-*tert*-butyl 3-ethyl isoxazolidine-2,3-dicarboxylate (i.e., **4**) is transferred to a pre-weighed 250-mL round-

bottomed flask using CH_2Cl_2, which is then evaporated (40 °C bath, 400–30 mmHg). The flask is equipped with a pre-weighed 4-cm oval Teflon-coated magnetic stirbar and dried while stirring on the vacuum pump (0.15 mmHg, 24 °C) for 10 h to provide a yellow oil (10.3 g). Column chromatography with 20% EtOAc in hexanes (Note 12) furnished **4** (6.35 g, 97.0 % purity, 64.9% based on compound **1**) as a clear yellow oil (Notes 13, 14, and 15).

B. *(S)-2-(tert-Butoxycarbonyl)isoxazolidine-3-carboxylic acid (**(S)-N-Boc-5-Oxaproline**) (**5**).* The oil **4** (6.35 g, 25.9 mmol, 1.0 equiv) is diluted with THF (95 mL) and transferred, using a long-stemmed plastic funnel, into a 500-mL, three-necked, round-bottomed flask equipped with a 4-cm Teflon-coated magnetic stir-bar, a 125-mL addition funnel, a thermometer fitted with a glass adaptor, and a nitrogen inlet. Tetrahydrofuran (10 mL) is used to rinse the flask and the remaining solution transferred into the reaction flask using a 10-mL pipette. The flask is cooled to 4 ºC using an ice-water bath. Using a 250-mL graduated cylinder, a solution of aqueous 1 M LiOH (103 mL, 103 mmol, 4.0 equiv) (Note 16), which had been chilled to 4 ºC in a refrigerator, is added to the addition funnel and is subsequently added to

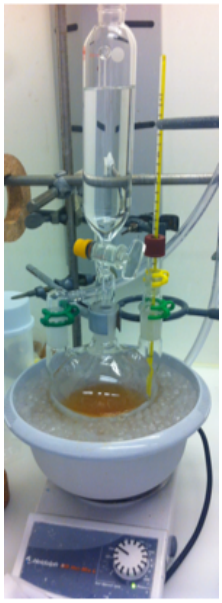


Figure 4. Glassware assembly for the hydrolysis reaction- Step B (picture obtained from submitters)

the flask dropwise over 45 min while maintaining an internal temperature of 3–4 °C (Figure 4). After the addition is complete, the funnel is removed and the flask is equipped with a glass stopper. The reaction mixture is stirred for 1.5 h at 24 °C and is monitored by TLC in 5% MeOH in CH_2Cl_2 using ninhydrin as stain (Note 17). After 1.5 h at 24 °C, TLC analysis indicates disappearance of starting material, and chloroform (250 mL) is added. The solution is acidified to pH 3 with an aqueous solution of 2 M $KHSO_4$ (100 mL). The mixture is poured into a 1-L separatory funnel. The aqueous layer is separated and washed with $CHCl_3$ (5 x 100 mL). The combined organic layers are dried over Na_2SO_4 (20 g) and filtered by suction using a fritted funnel (9 cm diameter, medium porosity). Chloroform (100 mL) is used to wash the Na_2SO_4, and the filtrate is concentrated by rotary evaporation into a 1-L round-bottomed flask (40 °C bath, 210–30 mmHg). The resulting pale yellow oil, containing (*S*)-2-(*tert*-butoxycarbonyl)isoxazolidine-3-carboxylic acid (i.e., **5**) is transferred to a 250-mL round-bottomed flask using CH_2Cl_2 which is evaporated (40 °C bath, 450–30 mmHg). A pre-weighed 4 cm Teflon-coated oval stir-bar is added, and the oily reside is stirred and dried on the vacuum pump (0.15 mmHg, 24 °C) for 18 h to afford a clear yellow oil **5** that solidifies upon standing (5.53 g, 25.4 mmol, 98% yield, 94.1% ee) (Notes 18, 19, 20, and 21).

Notes

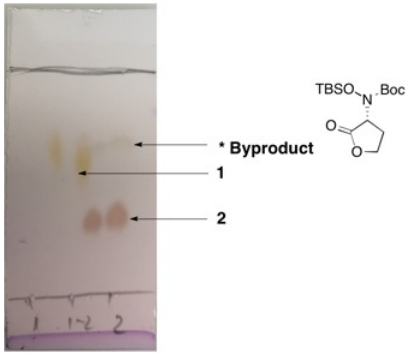
1. Prior to performing each reaction, a thorough hazard analysis and risk assessment should be carried out with regard to each chemical substance and experimental operation on the scale planned and in the context of the laboratory where the procedures will be carried out. Guidelines for carrying out risk assessments and for analyzing the hazards associated with chemicals can be found in references such as Chapter 4 of "Prudent Practices in the Laboratory" (The National Academies Press, Washington, D.C., 2011; the full text can be accessed free of charge at https://www.nap.edu/catalog/12654/prudent-practices-in-the-laboratory-handling-and-management-of-chemical). See also "Identifying and Evaluating Hazards in Research Laboratories" (American Chemical Society, 2015) which is available via the associated website "Hazard Assessment in Research Laboratories" at https://www.acs.org/content/acs/en/about/governance/committees

/chemicalsafety/hazard-assessment.html. In the case of this procedure, the risk assessment should include (but not necessarily be limited to) an evaluation of the potential hazards associated with methanol, sodium borohydride, acetonitrile, dry ice, ethyl acetate, hexanes, ninhydrin, ammonium chloride, sodium chloride, sodium sulfate, methylene chloride, triethylamine, sodium bicarbonate, methanesulfonyl chloride, tetrahydrofuran, tetrabutylammonium fluoride, diethyl ether,silica gel, lithium hydroxide, chloroform, and potassium bisulfate.

2. The protocol for preparing *(S)-ethyl 2-((tert-butoxycarbonyl)((tert-butyldimethylsilyl)oxy)amino)-4-oxobutanoate* (***1***) is described in *Org. Synth.* **2018**, *95*, 142-156.
3. The following reagents and solvents are used as received: The submitters purchased methanol from Sigma-Aldrich (chromasolv, ≥99.9%), while the checkers purchased methanol (99.9%) from Fisher Scientific. The submitters and checkers purchased the following chemicals: chloroform from Sigma-Aldrich, (chromasolv, ≥99.8%), diethyl ether (Sigma-Aldrich, ≥99.8%), dimethylformamide (Sigma-Aldrich, ≥99.8%), isopropyl alcohol (Sigma-Aldrich, ≥99.5%), triethylamine (Sigma-Aldrich, ≥99.5%), tetrabutylammonium fluoride (Sigma-Aldrich, 1.0 M in THF), sodium borohydride (Sigma-Aldrich, 98%), *N,N'*-diisopropylcarbodiimide (Sigma-Aldrich, 99%), 1-hydroxybenzotriazole hydrate (Sigma-Aldrich, ≥97%), sodium sulfate (Sigma-Aldrich, ≥99%), and lithium hydroxide monohydrate (Sigma-Aldrich, ≥98.5%). The submitters purchased tetrahydrofuran from Merck (for analysis, EMSURE), and the checkers purchased tetrahydrofuran from Fisher Scientific, (HPLC, >99.9%). The submitters purchased dichloromethane from Sigma-Aldrich (chromasolv, ≥99.5%) and the checkers purchased dichloromethane from Fisher Scientific (HPLC <99.9%). The submitters purchased methanesulfonyl chloride from Acros Organics (99.5%), and the checkers purchased from methanesulfonyl chloride Sigma-Aldrich (>99.7%). Potassium bisulfate (Fluka, 98%) was purchased by the submitters, while the checkers purchased the material from Oakwood Chemical (99%).The submitters used silica gel purchased from Fluka (high purity grade, pore size 60 Å, 230-400 mesh particle size) and the checkers purchased silica from EMD Millipore (60 Å, 230-400 mesh). Glass-backed extra hard layer TLC plates (60 Å (250 μm thickness containing F-254 indicator) were purchased by the submitters from Silicycle, and the checkers purchased TLC plates from EMD Millipore. The submitters purchased

Chloroform-d from Arma (99.8 atom% D), and the checkers purchased the material from Sigma-Aldrich (98.8% atom% D).1,3,5-Trimethoxybenzene (ABCR, 99%) was purchased by the submitters, while the checkers purchased the material from Sigma-Aldrich (>99 %). Deionized water is used throughout. The following salts are used as saturated aqueous solutions made by dissolving the salt in H_2O until saturation is reached: $NaHCO_3$ (Sigma-Aldrich, -40 +140 mesh, Na_2CO_3 2-5%), NaCl (ABCR, 99%), and NH_4Cl (Panreac Applichem, 99.5%).

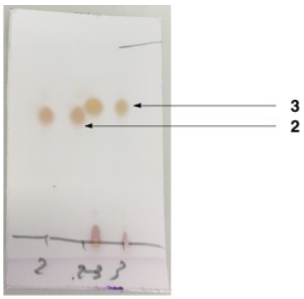
4. TLC of the crude alcohol **2** is monitored in 25% EtOAc in hexanes (stain with ninhydrin). (Product **2** has R_f = 0.33 and stains violet; starting material **1** has R_f = 0.65 and stains yellow). TLC data obtained from submitters.



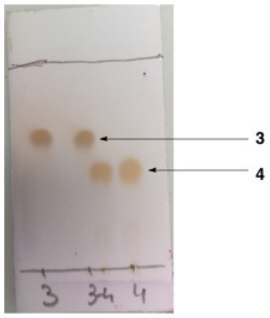
Byproduct (*) corresponds to the lactone. The ratio of byproduct to desired alcohol will be higher when the reaction is allowed to warm to room temperature for a long time or too much sodium borohydride is added at once.

5. Do not initiate the work-up procedure by evaporation of the MeOH from the reaction, since this reduces the yield and purity of the final compound. The work-up procedure described above should be followed.
6. Reactions 1, 2 and 3 in Step A should be done in a timely fashion, as intermediates are not very stable and will start to decompose over a couple days. Checkers consistently obtained a yield of <45 % when Step A was done over the period of 5 days. In particular, crude mesylate **3** should be taken to the next step immediately upon concentration. The checkers observed significant darkening of the mesylate (**3**) on stirring under high vacuum over the course of 3 h.

7. The identify of product **2** was determined, as follows. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.17–0.20 (m, 6 H), 0.92 (s, 9 H), 1.27 (t, J = 7.1 Hz, 3 H), 1.48 (s, 9 H), 2.09–2.28 (m, 2 H), 2.32 (t, J = 6.1 Hz, 1 H), 3.72–3.78 (m, 2 H), 4.11–4.30 (m, 2 H), 4.45 (dd, J = 8.9, 5.7 Hz, 1 H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: –4.9, –4.7, 14.1, 17.9, 25.8, 28.1, 31.4, 59.7, 61.2, 63.2, 82.3, 157.8, 170.1. HRMS (ESI) calcd. for $C_{17}H_{36}NO_6Si$ $[M+Na]^+$ 400.2131, found 400.2136.
8. The mesylation reaction is monitored by TLC with 40% EtOAc in hexanes (stain with ninhydrin). Product **3** has R_f = 0.65 and starting material **2** has R_f = 0.57. TLC data obtained from submitters.

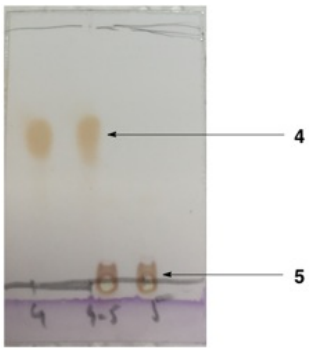


9. The identity of product **3** is confirmed by the following characterization data. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.13–0.20 (m, 6 H), 0.92 (s, 9 H), 1.27 (t, J = 7.1, 1.0 Hz, 3 H), 1.48 (s, 9 H), 2.17–2.35 (m, 1H), 2.35–2.51 (m, 1 H), 3.01 (s, 3 H), 4.11–4.30 (m, 1H), 4.30–4.48 (m, 2 H), 4.48–4.56 (m, 1 H). ^{13}C NMR (101 MHz, $CDCl_3$) δ: 14.9, 28.3, 33.1, 59.6, 61.8, 68.5, 82.7, 156.0, 170.8. HRMS (ESI) calcd. for $C_{18}H_{37}NO_8SSi$ $[M+Na]^+$ 478.1907, found 478.1915.
10. The cyclization reaction is monitored by TLC with 40% EtOAc in hexanes (stain with ninhydrin). Product **4** has R_f = 0.45 and stains yellow and starting material **3** has R_f = 0.65 and stains brown). TLC data obtained from submitters.

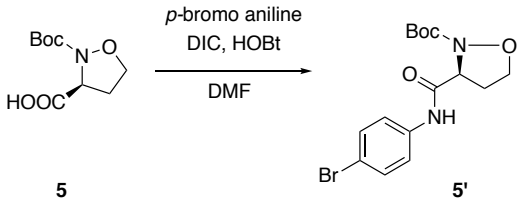


11. The checkers observed incomplete cyclization (ca. 50% conversion of **3**) after the addition of 1.5 equivalents TBAF solution. Dropwise addition of an additional 0.25 equivalents TBAF (1.0 M solution in THF, 10 mL) over the course of 5 minutes led to complete conversion **3** within 10 min; the reaction was quenched and worked up at this point.
12. The column (8 x 30 cm) was packed with 450 g of silica gel. The silica gel was loaded in 20% EtOAc in hexanes (~1 L). The crude material was dissolved in 10 mL of the eluent and loaded onto the silica gel. The flask is washed with 10 mL eluent in order to ensure that no product remains on the side of the flask, and the eluent added to the column. Sand (300 g) (~1.5 cm) is carefully added to the top of the column. No pressure is applied. Elution is performed with 20% EtOAc in hexanes and fractions collected in 50-mL tubes after one column volume had eluted. The desired product was obtained in fractions 30–53. The fractions containing the desired product were concentrated by rotary evaporation (35 °C bath, 340–8 mmHg).
13. ^{1}H-NMR, ^{13}C-NMR and HRMS confirm the purity of product **4** and match literature values.[1] ^{1}H NMR (400 MHz, Chloroform-*d*): δ 4.68 (dd, *J* = 9.4, 4.8 Hz, 1 H), 4.23 (q, *J* = 7.1 Hz, 2 H), 4.18 – 4.07 (m, 1 H), 3.91 – 3.75 (m, 1 H), 2.71 – 2.53 (m, 1 H), 2.55 – 2.39 (m, 1 H), 1.50 (s, 9 H), 1.30 (t, *J* = 7.1 Hz, 3 H). ^{13}C NMR (151 MHz, Chloroform-*d*): δ 170.8, 155.9, 82.7, 68.4, 61.8, 59.6, 33.1, 28.3, 14.2. HRMS (ESI) calcd. for $C_{11}H_{20}NO_5$ $[M+Na]^+$ 268.1161, found 238.1159.
14. The purity of the compound was calculated by qNMR with a delay of relaxation of 30 seconds, using 17.1 mg of 1,3,5-trimethoxybenzene (purity ≥99%) and 21.9 mg of the compound **4**.
15. A second run performed at the same scale provided 6.60 g (67%) of the identical product (**4**).

16. The LiOH solution (1 M) is prepared by dissolving 42 g LiOH monohydrate in 1000 mL deionized water.
17. TLC of the hydrolysis reaction is monitored in 5% MeOH in CH_2Cl_2 (stain with ninhydrin). TLC data obtained from submitters.



18. The identify of product **5** was characterized with the following data, which matched the literature values.[2] ^{1}H NMR (600 MHz, $CDCl_3$) δ: 1.44 (s, 9H**),** 2.43 – 2.54 (m, 1H), 2.54–2.66 (m, 1H), 3.67–3.91 (m, 1H), 3.98 – 4.20 (m, 1H), 4.67 (dd, *J* = 9.5, 4.9 Hz, 1H), 10.53 (s, 1H). ^{13}C NMR (151 MHz, $CDCl_3$) δ: 28.2, 32.9, 59.6, 68.6, 83.5, 156.2, 174.9. HRMS (ESI) calcd. for $C_9H_{15}NO_5$ $[M+Na]^+$ 240.0848, found 240.0849. The purity of the compound was calculated by qNMR with a delay of relaxation of 30 sec, using 10.40 mg of 1,3,5-trimethoxybenzene (purity ≥99%) and 14.41 mg of the compound **5**.
19. A second run performed at the same scale provided 5.26 g (94%) of the identical product (**5**).
20.



5 **5'**

Derivatization of *(S)-N*-Boc-5-Oxaproline

For the determination of the enantiomeric excess of the final compound a derivatization is required for a more accurate result. To *(S)-N*-Boc-5-oxaproline **5** (10.0 mg, 46 μmol, 1.0 equiv) in DMF (200 μL) are added

N,N'-diisopropylcarbodiimide (7.2 μL, 47 μmol, 1.0 equiv) and 1-hydroxybenzotriazole hydrate (7.1 mg, 46 μmol, 1.0 equiv) and the solution is stirred for 2 min. para-Bromoaniline (8.4 mg, 49 μmol, 1.1 equiv) is added to this mixture in one portion, and the reaction is stirred for 2 h at room temperature. The mixture is diluted with CH_2Cl_2 (3 mL) and washed with H_2O (3 mL), brine (3 mL), dried over Na_2SO_4, filtered and concentrated. The solution is loaded on silica and the product is isolated by column chromatography using a gradient of 10–40% EtOAc in hexanes (8.7 mg, 51% yield, 94.1% ee). ^{1}H NMR (600 MHz, $CDCl_3$) δ: 1.54 (s, 9H), 2.59–2.75 (m, 2H), 3.73–3.91 (m, 1H), 4.07–4.23 (m, 1H), 4.77–4.89 (m, 1H), 7.39–7.51 (m, 4H), 8.49 (s, 1H); **^{13}C NMR** (151 MHz, $CDCl_3$) δ: 28.2, 32.5, 62.8, 69.5, 84.2, 117.3, 121.5, 132.1, 136.4, 157.8, 168.5; **HRMS (ESI)** calcd. for $C_{15}H_{20}BrNO_4$ $[M+Na]^+$ 393.0426, found 393.0429. In order to prepare a racemic sample of **5′**, racemic **1** was prepared as described in *Org. Synth.* **2018**, *95*, 142-156 and taken through an analogous procedure for that used to prepare (*S*)-**5′** from (*S*)-**1**.

21. Enantiomeric excess of the enantiomeric amides (94%) was determined by chiral HPLC. Separation was performed by HPLC on a Chiralcel IA column using hexanes/isopropyl alcohol (8:2), 25 °C, with a flow rate of 1.0 mL/min, while monitoring at 210 nm. Retention time (t_R) of the minor enantiomer = 8.58 min, and retention time (t_R) of the major enantiomer = 15.39 min.

Working with Hazardous Chemicals

The procedures in *Organic Syntheses* are intended for use only by persons with proper training in experimental organic chemistry. All hazardous materials should be handled using the standard procedures for work with chemicals described in references such as "Prudent Practices in the Laboratory" (The National Academies Press, Washington, D.C., 2011; the full text can be accessed free of charge at http://www.nap.edu/catalog.php?record_id=12654). All chemical waste should be disposed of in accordance with local regulations. For general guidelines for the management of chemical waste, see Chapter 8 of Prudent Practices.

In some articles in *Organic Syntheses*, chemical-specific hazards are highlighted in red "Caution Notes" within a procedure. It is important to

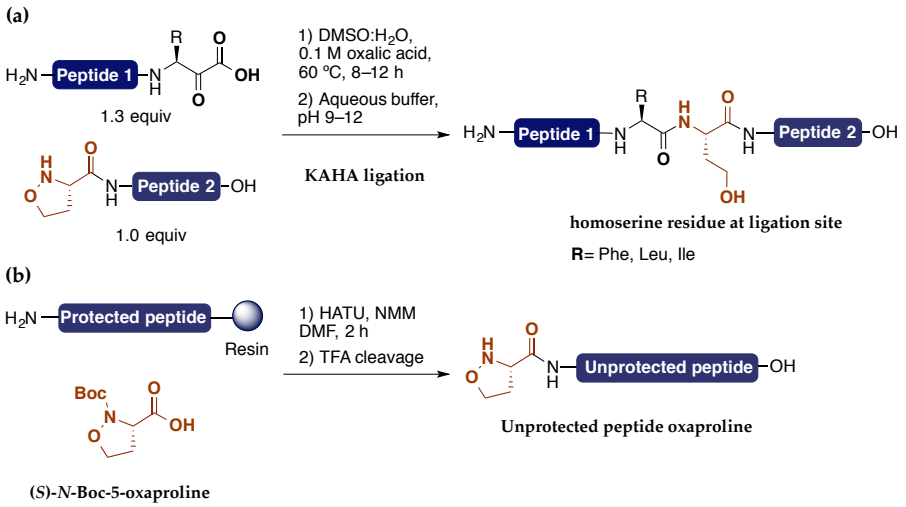
recognize that the absence of a caution note does not imply that no significant hazards are associated with the chemicals involved in that procedure. Prior to performing a reaction, a thorough risk assessment should be carried out that includes a review of the potential hazards associated with each chemical and experimental operation on the scale that is planned for the procedure. Guidelines for carrying out a risk assessment and for analyzing the hazards associated with chemicals can be found in Chapter 4 of Prudent Practices.

The procedures described in *Organic Syntheses* are provided as published and are conducted at one's own risk. *Organic Syntheses, Inc.*, its Editors, and its Board of Directors do not warrant or guarantee the safety of individuals using these procedures and hereby disclaim any liability for any injuries or damages claimed to have resulted from or related in any way to the procedures herein.

Discussion

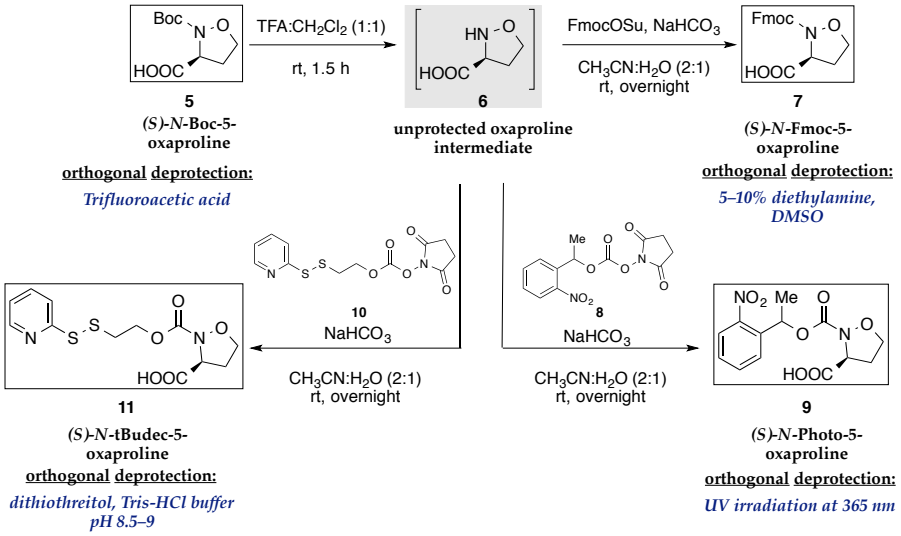
As part of an effort to contribute to the field of chemical ligation[2] we reported in 2012 the α-ketoacid–hydroxylamine ligation (KAHA ligation) with (*S*)-*N*-Boc-5-oxaproline. This reaction makes possible the chemical synthesis of proteins from unprotected peptide segments.[3] KAHA ligation with 5-oxaproline leads to a depsipeptide ester which rearranges to the corresponding amide in basic buffers generating a homoserine residue at the ligation site (Figure 1a).[4] The utility of the KAHA ligation has been illustrated in the synthesis of Pup, CspA, UFM1, SUMO2, SUMO3, betatrophin, irisin and IFITM3 proteins[3,5] and can be considered as a complementary method to native chemical ligation (NCL).[6]

Unlike NCL, the KAHA ligation does not use an *N*-terminal cysteine residue and *C*-terminal thioester or thioester surrogate.[5] (*S*)-*N*-Boc-5-oxaproline presents high stability to acidic cleavage conditions of the peptide from resin and reactivity to the α-ketoacid functionality. It is manually coupled to the resin containing the peptide fragment using standard coupling conditions and does not affect any aspect of the peptide synthesis or purification (Figure 1b).



Scheme 1. (a) General description of the KAHA ligation. (b) Coupling of (*S*)-*N*-Boc-5-oxaproline on protected peptide segment followed by cleavage of peptide fragment from resin.

It is important to note that this building block can be readily converted to orthogonal (*S*)-*N*-protected-5-oxaprolines through the free-hydroxylamine intermediate **6** (Figure 2). This is significant for the use of sequential KAHA ligations for the synthesis of small and medium-size proteins.[3,5]



Scheme 2. Conversion from *(S)-N*-Boc-5-oxaproline to orthogonal protected oxaprolines.

Our original approach to the synthesis of (*S*)-*N*-Boc-5-oxaproline was based on a modified procedure of Vasella et al[8] and utilized a [3+2] cycloaddition with ethylene in a pressurized reactor. Although the protocol is well established it requires an expensive chiral auxiliary and affords a 6:4 diastereoselectivity ratio after the cycloaddition reaction. The two diastereoisomers can be easily separated by two recrystallizations but with relatively poor recovery.[3] Importantly, the requirement of using a pressurized reactor created a major bottleneck for preparing this building block and using KAHA ligation as an alternative for the chemical synthesis of peptides or proteins.

This encouraged us to enable an efficient, economical route to enantiopure (*S*)-*N*-Boc-5-oxaproline. We describe here a scalable and practical route to the synthesis of (*S*)-*N*-Boc-5-oxaproline. The route begins with the sodium borohydride reduction of *(S)-ethyl 2-((tert-butoxycarbonyl)((tert-butyldimethylsilyl)oxy)amino)-4-oxobutanoate*[9] ***1*** to obtain alcohol **2**. Following the mesylation of the alcohol **2** and the one-pot TBS deprotection-cyclization we subsequently formed the cyclic hydroxylamine **4**. Hydrolysis of the ethyl ester afforded the (*S*)-*N*-Boc-5-oxaproline building block **5** with 95% ee and good overall yield.

The applicability of the newly developed route to the (*S*)-*N*-Boc-5-oxaproline has been showcased in the synthesis of SUMO2, SUMO3, betatrophin and irisin proteins.[5] In summary we have developed a scalable and practical multistep synthesis of the oxaproline building block.

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Appendix
Chemical Abstracts Nomenclature (Registry Number)

Sodium borohydride; (16940-66-2)
Methanesulfonyl chloride; (124-63-0)

Triethylamine; (121-44-8)
Tetrabutylammonium fluoride; (429-41-4)
Lithium hydroxide monohydrate; (1310-66-3)

Claudia Murar was born in Deva, Romania in 1988. She moved for her studies to France where she studied chemistry at the National Institute of Applied Sciences (INSA), Rouen (France). She worked for one year at GSK, King of Prussia (USA) after which she completed her master thesis at Novartis, Basel (Switzerland). She joined the group of Prof. Jeffrey Bode at ETH Zurich in 2012 for her Ph.D. Her research focuses on the total chemical synthesis of hormone proteins and therapeutic proteins, development of new building blocks for chemical ligation and synthesis of unnatural amino acids.

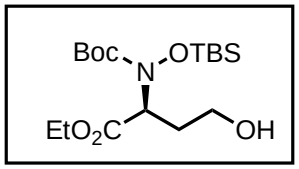
Thibault Harmand studied Chemistry at the University of Nantes and Lyon (France). After his master thesis with Prof. George Fleet at the University of Oxford, he joined the group of Prof. Jeffrey Bode at ETH Zurich. His research focuses on the total chemical synthesis of proteins such as the hormone protein betatrophin and the antiviral membrane protein IFITM3 as well as the development of amino acids building blocks for chemical ligation.

Hikaru Takano was born in Nagasaki, Japan in 1989. He received his B.Eng. degree in 2012 from Saitama University (Prof. Katsukiyo Miura). He then moved to Tokyo Medical and Dental University and is currently pursuing his Ph.D. degree under the supervision of Prof. Hirokazu Tamamura (2012-present). His current research focuses on the development of chemical probes, especially fluorescent dyes, photolabile protecting groups and caged compounds.

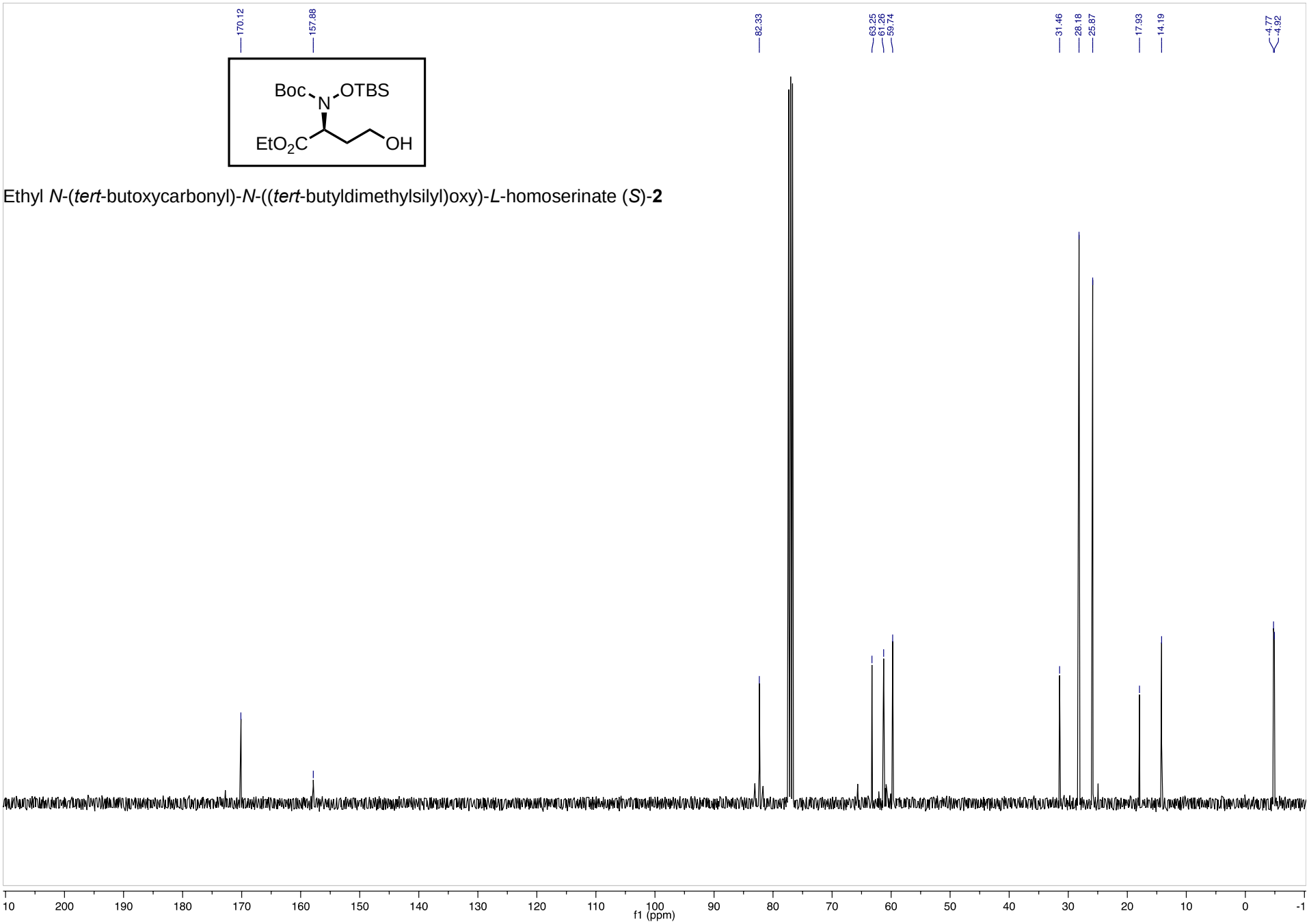
Jeffrey Bode is Professor of Synthetic Organic Chemistry at ETH Zürich. In addition, he serves as an Executive Editor for the *Encyclopedia of Reagents for Organic Synthesis*, co-Editor in Chief of *Helvetica Chimica Acta*, and a Principal Investigator at the *Institute of Transformative bio-Molecules (ITbM)* at Nagoya University in Japan. His research group focuses on the development of new reactions, including methods for N-heterocycles, chemical protein synthesis, bioconjugation, and chemical biology.

Jacob C. Timmerman graduated from the University of North Carolina at Chapel Hill in 2012 with a B.A. in Chemistry. Later in 2012, Jacob began his graduate studies at Duke University under the advisement of Professor Ross A. Widenhoefer where his research focused on the development and mechanistic studies of gold(I)-catalyzed hydrofunctionalization reactions of alkenes. After completing his Ph.D. in 2017, Jacob joined the laboratories of Professor John L. Wood as a postdoctoral research associate where his research focuses on the total synthesis of natural products.

Yu-Wen Huang was born in Hsinchu, Taiwan (R.O.C.) in 1982. He received his bachelor's degree from National Cheng Kung University in 2005. He then joined the M.S. program at the National Tsing Hua University working under the supervision of Professor Shang-Cheng Hung on carbohydrate synthesis. In 2016, he received his Ph.D. degree from the University of Rochester where he worked with Professor Alison J. Frontier on 1,6-conjugate addition initiated Nazarov reactions and sequential 1,5-hydride transfer chemistry. He is currently a post-doctoral fellow in the CPRIT lab (Baylor University) with Professor John L. Wood working on the total synthesis of natural products.



Ethyl *N*-(*tert*-butoxycarbonyl)-*N*-((*tert*-butyldimethylsilyl)oxy)-*L*-homoserinate (*S*)-**2**



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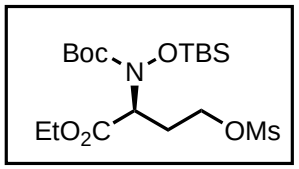

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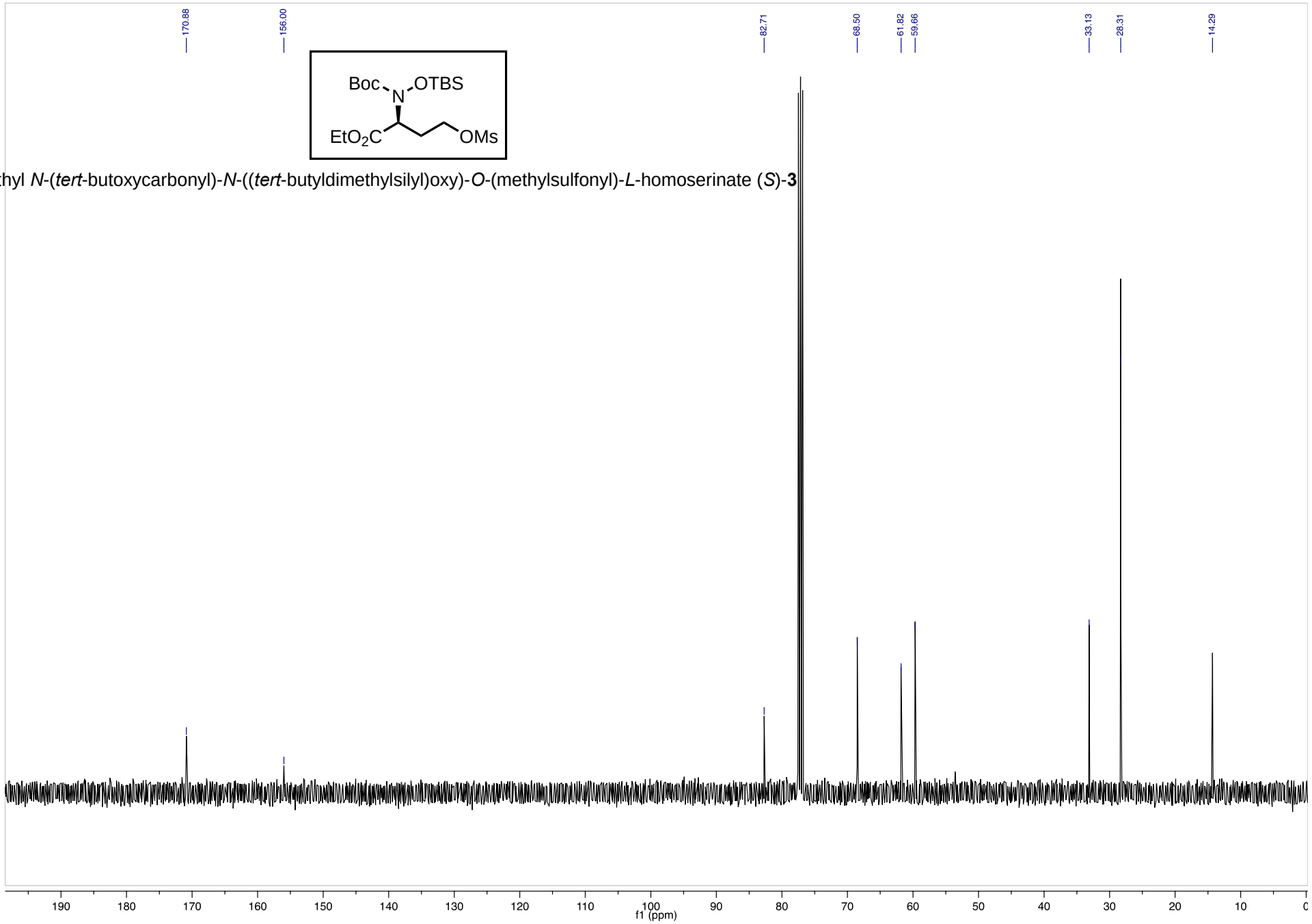
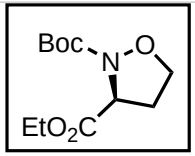
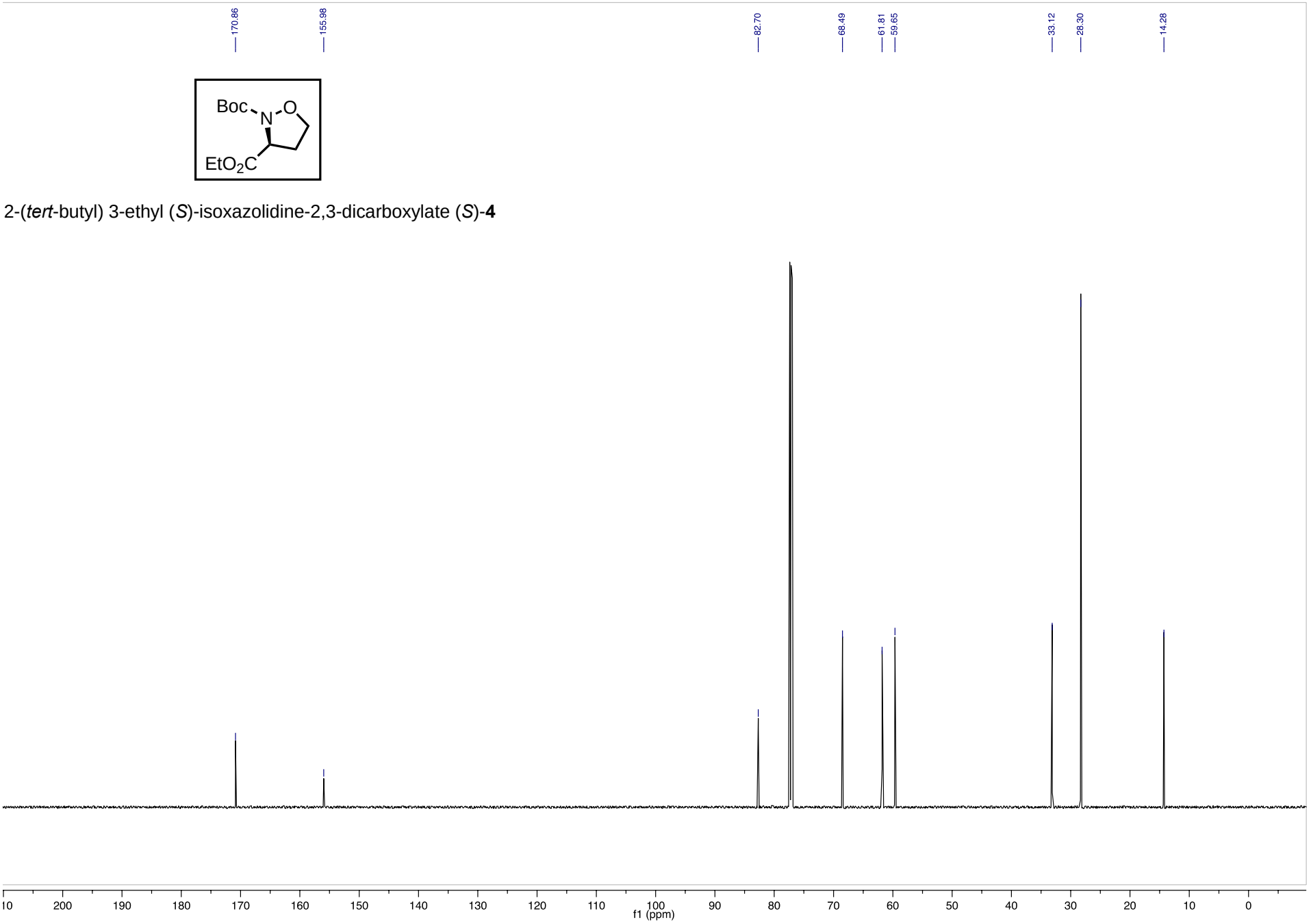


Ethyl *N*-(*tert*-butoxycarbonyl)-*N*-((*tert*-butyldimethylsilyl)oxy)-*O*-(methylsulfonyl)-*L*-homoserinate (*S*)-**3**



2-(*tert*-butyl) 3-ethyl (*S*)-isoxazolidine-2,3-dicarboxylate (*S*)-**4**



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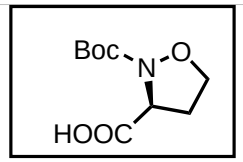
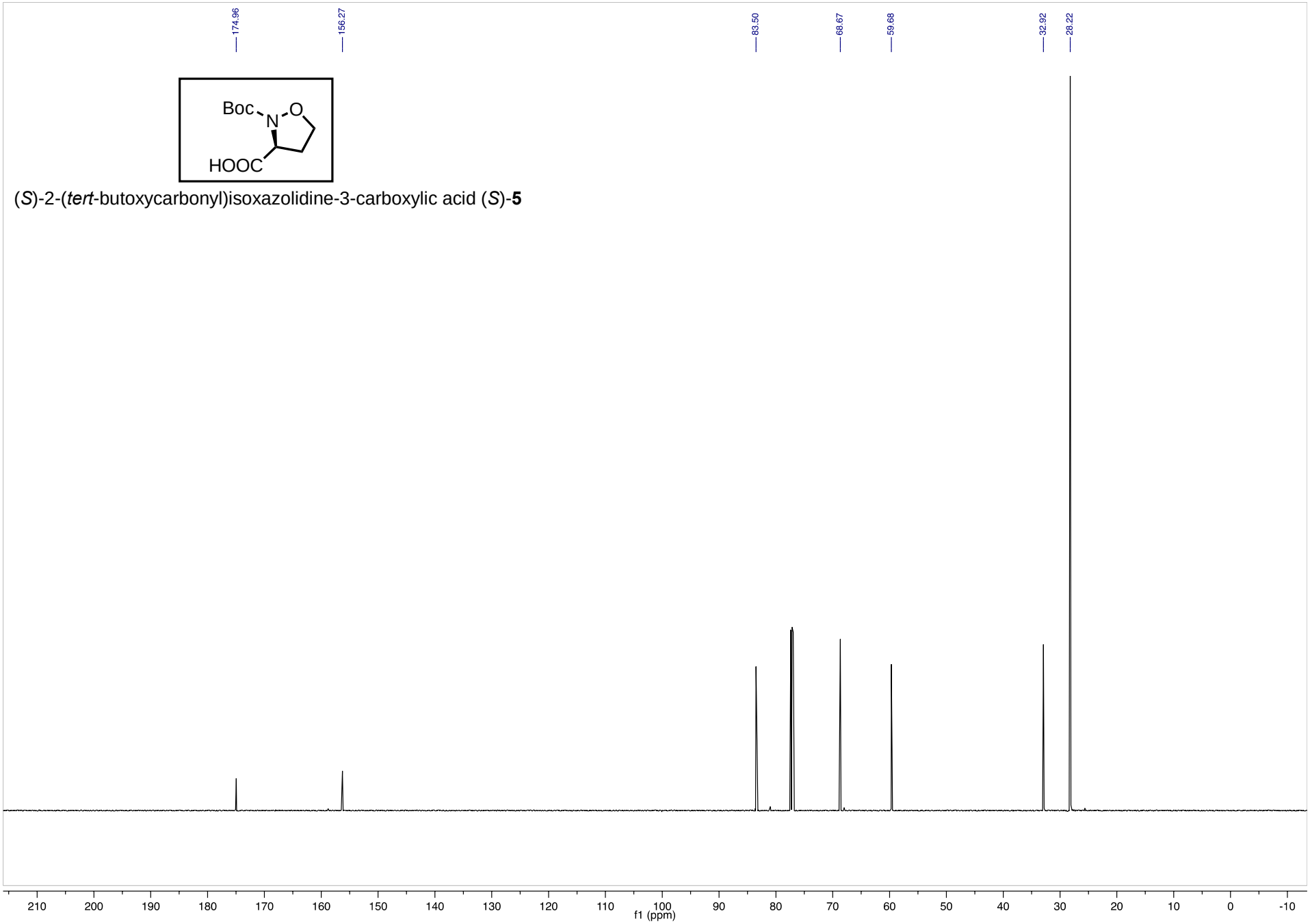


(*S*)-2-(*tert*-butoxycarbonyl)isoxazolidine-3-carboxylic acid (*S*)-**5**



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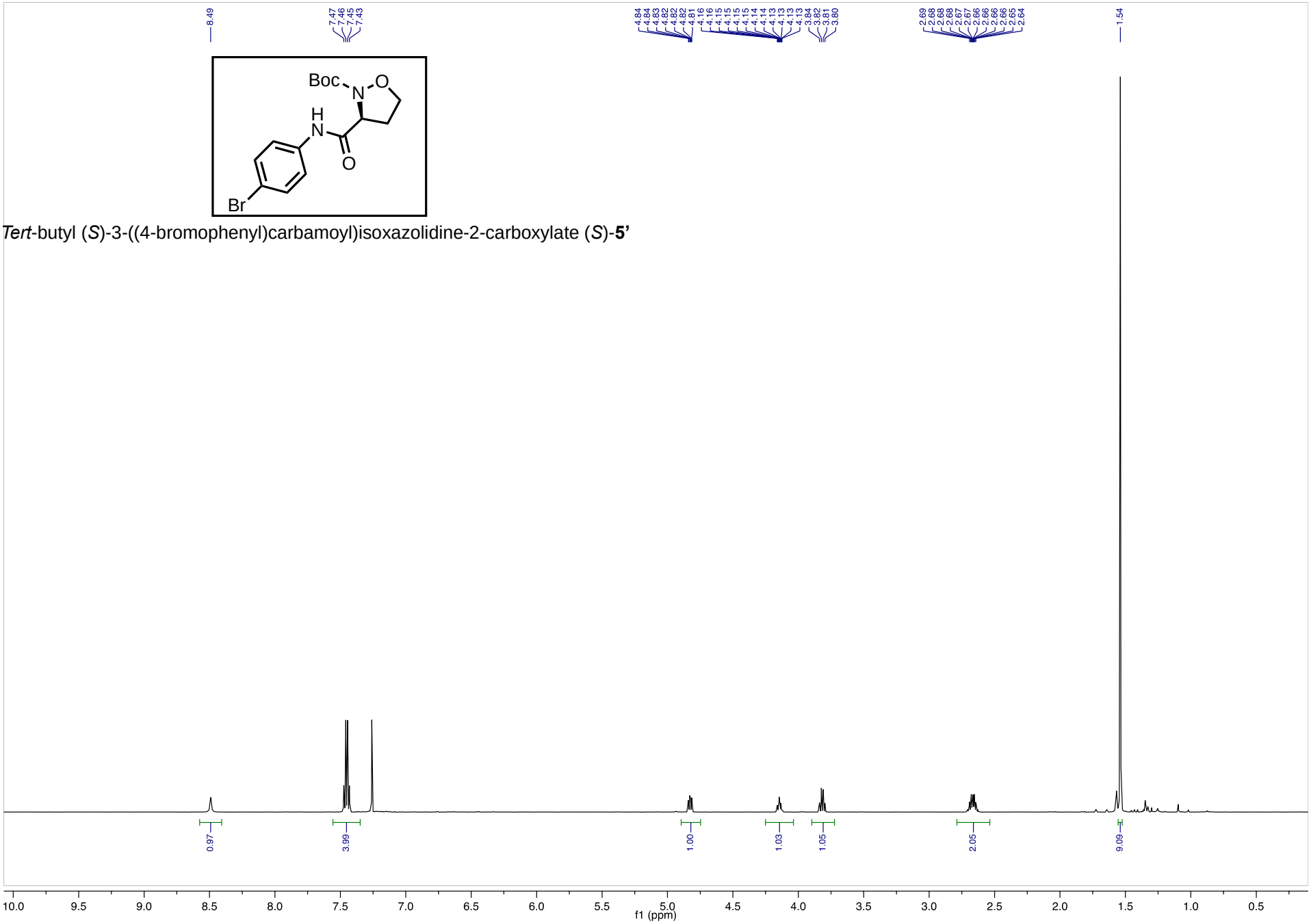

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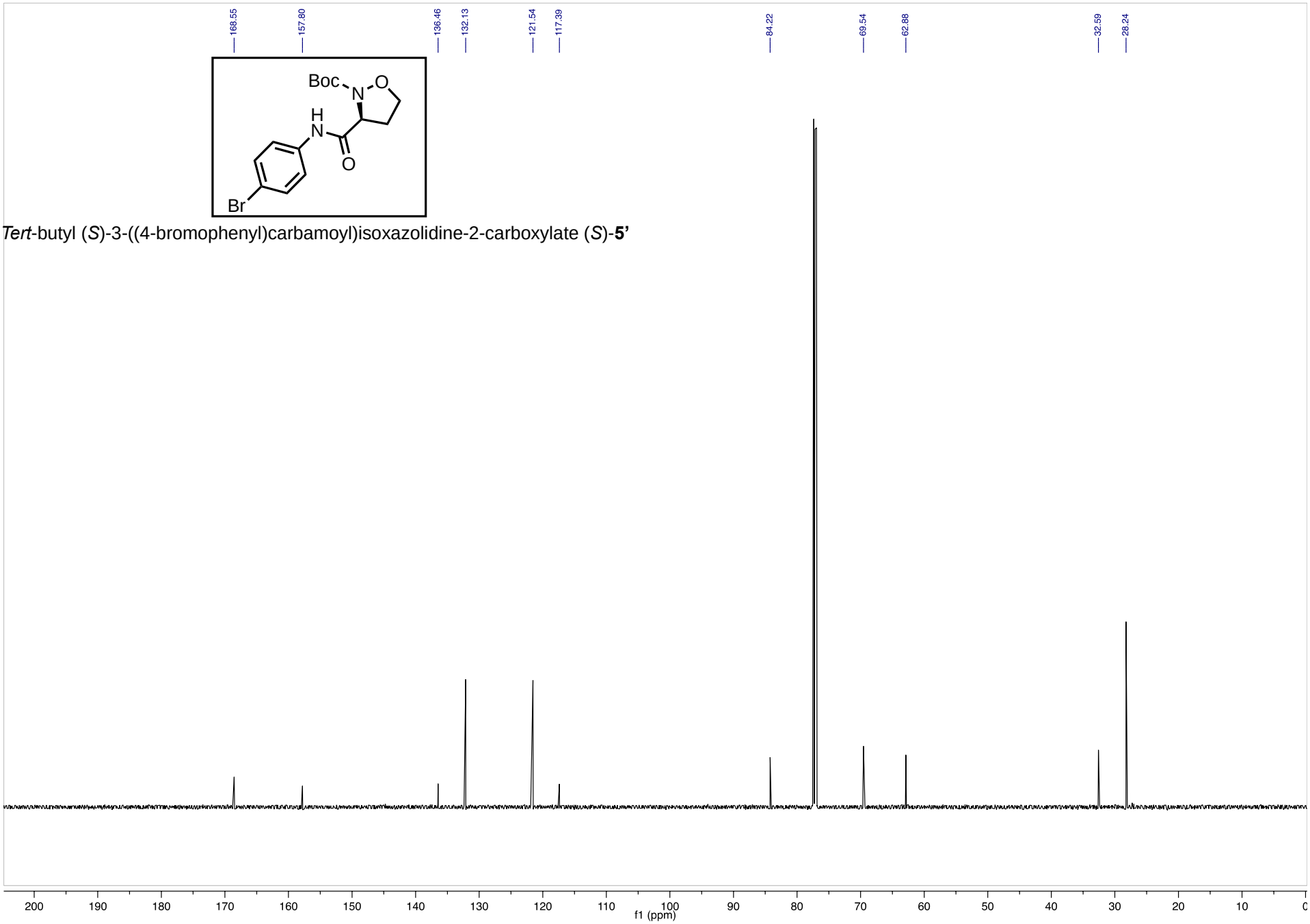

Tert-butyl (*S*)-3-((4-bromophenyl)carbamoyl)isoxazolidine-2-carboxylate (*S*)-**5'**