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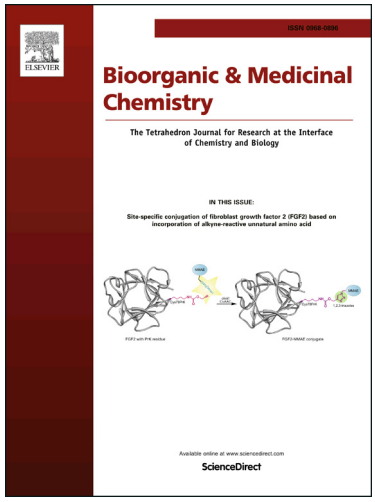

Improved synthesis of (*S*)-*N*-Boc-5-Oxaproline for protein synthesis with the α-ketoacid-hydroxylamine (KAHA) ligation

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Graphical Abstract

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Improved Synthesis of (*S*)-*N*-Boc-5-Oxaproline for Protein Synthesis with the α-Ketoacid-Hydroxylamine (KAHA) Ligation

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Improved synthesis of (*S*)-*N*-Boc-5-Oxaproline for protein synthesis with the α-ketoacid-hydroxylamine (KAHA) ligation

Claudia E. Murar [a], Thibault J. Harmand [a] and Jeffrey W. Bode [a,b]*

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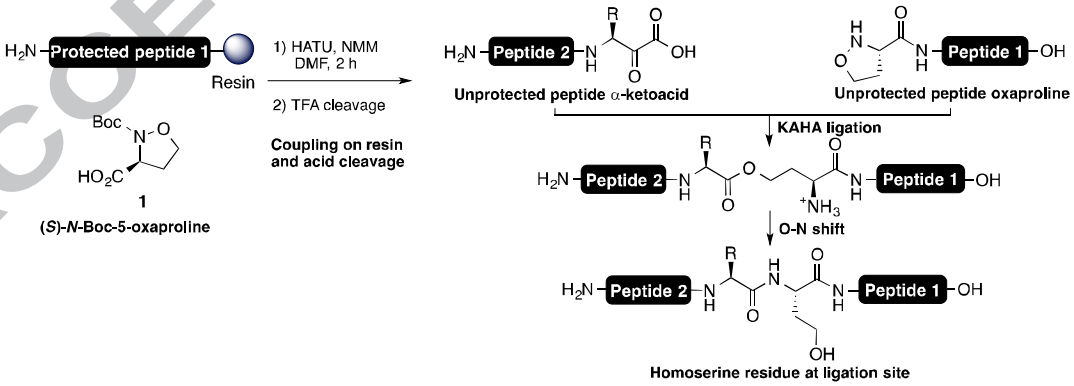
ABSTRACT

We describe a new route for the synthesis of (*S*)-*N*-Boc-5-oxaproline. This building block is a key element for the chemical synthesis of proteins with the α-ketoacid-hydroxylamine (KAHA) ligation. The new synthetic pathway to the enantiopure oxaproline is based on a chiral amine mediated enantioselective conjugate addition of a hydroxylamine to *trans*-4-oxo-2-butenoate. This route is practical, scalable and economical and provides decagram amounts of material for protein synthesis and conversion to other protected forms of (*S*)-oxaproline.

1. Introduction

As part of our effort to establish a new platform for the chemical synthesis of proteins using Fmoc-SPPS, we reported in 2012 the α-ketoacid–hydroxylamine ligation (KAHA ligation) with (*S*)-*N*-Boc-5-oxaproline ((*S*)-*N*-5-Opr).[1] The KAHA ligation enables coupling of an unprotected C-terminal peptide α-ketoacid with a segment bearing an N-terminal 5-oxaproline (5-Opr). The initially formed depsipetide product readily rearranges to the corresponding backbone amide in basic buffers by an O-N shift, generating a homoserine residue at the ligation site (Scheme 1).[2] The C-terminal peptide α-ketoacids are readily prepared using a variety of solid supported linkers and we have reported KAHA ligations with Leu, Phe, Val, Ile, Glu, Arg, Tyr, and Glu-derived α-ketoacids. The use of hydroxylamines derived from amino acids, however, is plagued by poor stability of the unprotected forms.[3,4]



Scheme 1. Coupling of (*S*)-*N*-Boc-5-oxaproline ((*S*)-*N*-Boc-5-Opr) onto a protected peptide segment and acidic cleavage from the resin, followed by KAHA ligation

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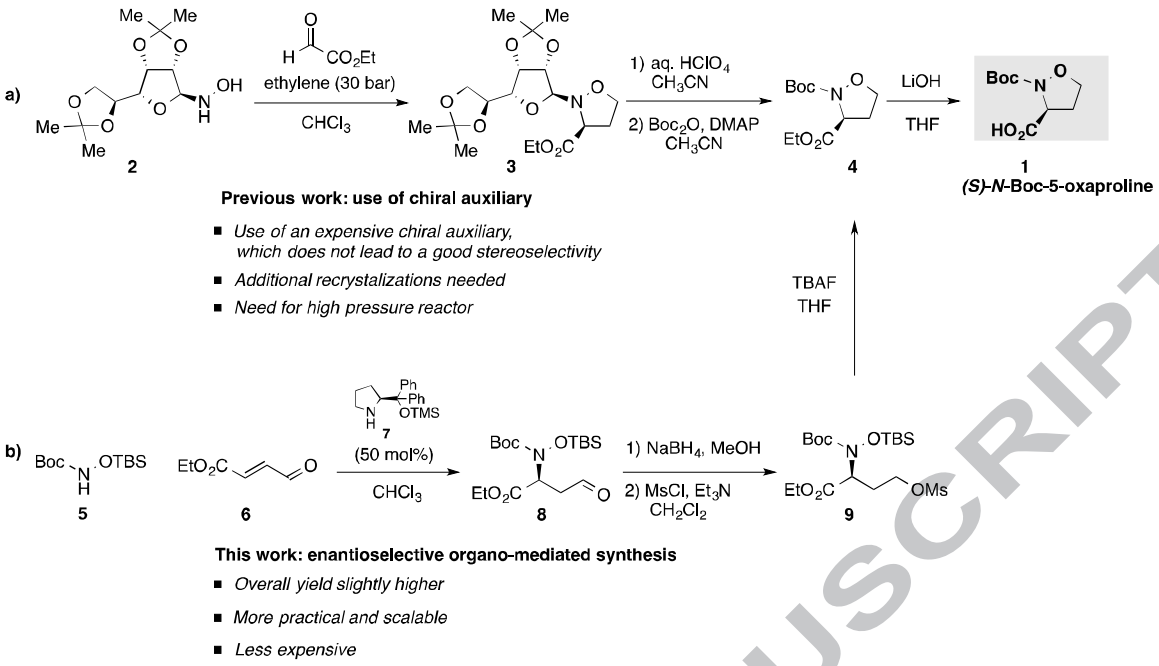


Scheme 2. a) Prior route to the synthesis of (S)-*N*-Boc-5-oxaproline. b) New route for the preparation of (S)-*N*-Boc-5-oxaproline.

To address this, we identified a number of cyclic hydroxylamine derivatives as being both chemically stable to standard Fmoc-SPPS and excellent performance in the KAHA ligation (Scheme 1). (*S*)-*N*-Boc-5-oxaproline has been utilized in the synthesis of SUMO2, SUMO3,[5] irisin,[6] betatrophin,[7] AS48,[8] and NP4.[9] This building block can be readily converted to orthogonally protected (*S*)-*N*-protected-5-oxaprolines, which are used for sequential KAHA ligations in the synthesis of small and medium-size proteins.

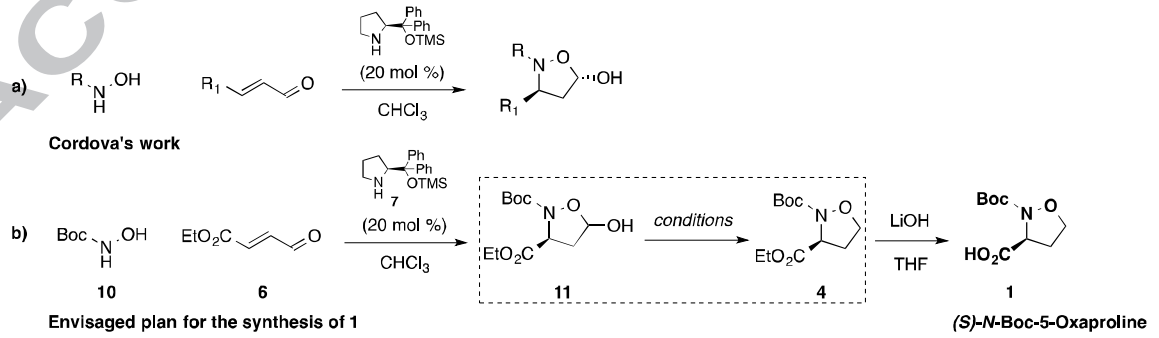
Our efforts to expand the use of KAHA ligation for the chemical synthesis of peptides and proteins required a scalable and practical synthesis for the key monomer *(S)-N*-Boc-5-Opr. Our original approach to the synthesis of *(S)-N*-Boc-5-Opr was based on a modified procedure of Vasella *et al.*[10] and utilized a [3+2] cycloaddition with ethylene in a pressurized reactor (Scheme 2a). However, this protocol required an expensive chiral auxiliary that afforded a 6:4 diastereomeric ratio after the cycloaddition reaction. After several recrystallizations the two diastereoisomers can be separated but with relatively poor recovery.[1] Above all, the requirement of a pressurized reactor in this procedure is a major impediment for preparing this building block in a practical way.

In this report we document our development of a new synthetic pathway for the synthesis of *(S)-N*-Boc-5-Opr based on an enantioselective organomediated reaction (Scheme 2b). This procedure can be used to easily prepare >5 g of *(S)-N*-Boc-5-Opr in a research lab and has been employed by a research partner for the production of >1 kg of this building block.

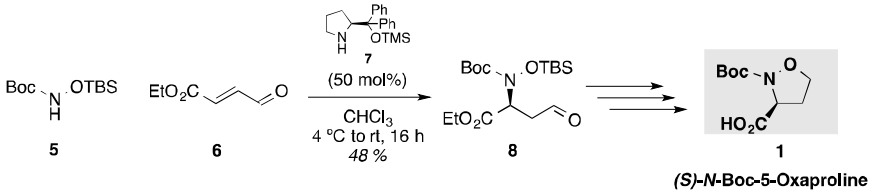
2. Enantioselective organomediated route for the synthesis of *(S)-N*-Boc-5-Oxaproline

2.1. Route 1: from 5-hydroxyisoxazolidine

Inspired by the work of Cordova on asymmetric tandem reaction between *N*-protected hydroxylamines and α□β-unsaturated aldehydes catalyzed by diarylprolinols. We envisaged a route for the synthesis of *(S)-N*-Boc-5-Opr from the corresponding 5-hydroxyisoxazolidine in three steps (Scheme 3).[11]



Scheme 3. a) Cordova's work to substituted 5-hydroxyisoxazolidines. b) Our envisaged route for the preparation of (*S*)-*N*-Boc-5-Opr inspired from the work of Cordova.



Scheme 4. Conjugate addition of silyl protected hydroxylamines to α,β-unsaturated aldehydes.

Substituted 5-hydroxyisoxazolidine **11** was readily prepared from *N*-Boc-protected hydroxylamine and ethyl *trans*-4-oxo-2-butenoate catalyzed by TMS protected diphenylprolinol in a 3:1 diastereomeric ratio. The synthesis of the TMS protected diphenylprolinol catalyst was straightforward starting from the commercially available, affordable intermediate (*S*)-diphenyl(pyrrolidin-2-yl)methanol. Otherwise, this catalyst is commercially available from a great number of vendors. The key step in this route was the mild reduction of the cyclic hydroxylamine **11**. We therefore probed conditions for this step. Mild activation of the cyclic hydroxylamine **11** with Shoda's catalyst[12–14] followed by reduction with sodium borohydride led to decomposition of the activated adduct as indicated by ^{1}H NMR. Treatment with methanesulfonyl chloride and acetic anhydride under basic conditions or with triphenylphosphine under basic conditions failed to give the activated intermediate validating the low stability of this intermediate. We consequently turned our attention to the use of Lewis acids in the presence of reducing agents but both $InCl_3$ and $TiCl_4$ entailed significant degradation of starting material. Silane reduction using $BF_3{\cdot}Et_2O$ as Lewis acid or in acidic conditions with TFA were also unfruitful. Ultimately, attempts of reduction with borohydrides were deemed unproductive.

Although the conditions described above are well established for cyclic hemiacetals,[15–17] these were unsuccessful for our cyclic hydroxylamine-hemiacetal. Therefore we turned towards the preparation of Boc-Opr using an asymmetric addition of silyl-protected hydroxylamines to α□β-unsaturated aldehydes, followed by cyclization.

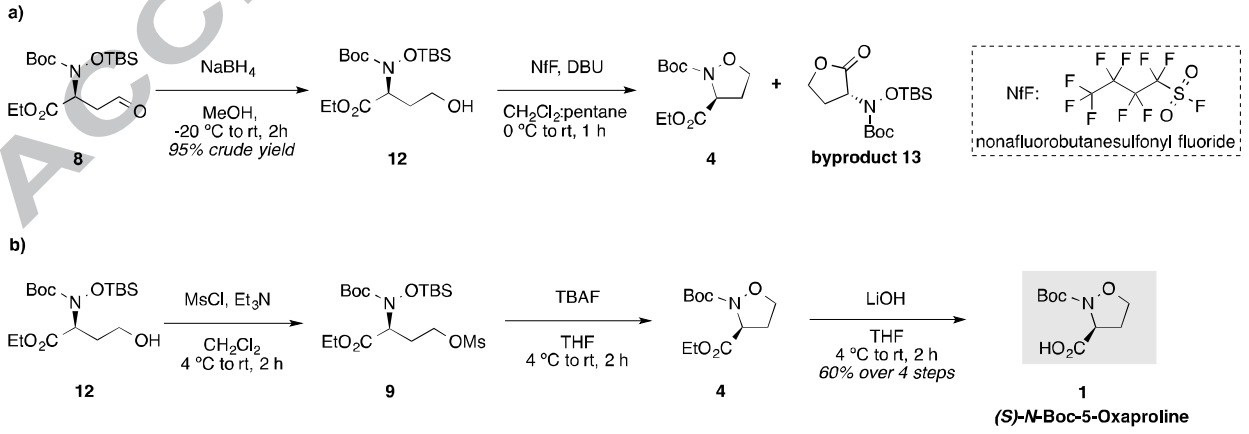
2.2. Route 2: from β-amino aldehydes

In the next strategy we first examined literature examples based on the extensive work of Cordova and MacMillan in the field of conjugate addition reactions involving *N*-centered nucleophiles and chiral amine catalysts.[11,18] Both groups documented the asymmetric addition of silyl protected hydroxylamines to α□β-unsaturated aldehydes in the presence of a chiral catalyst and we turned our attention to a modified synthetic protocol (Scheme 4).[19,20]

We proceeded with the TMS catalyst **7** that gave the addition product **8** in a 96:4–98:2 er.[21] Evaluation of the conditions for the conjugate addition revealed that using 50 mol% of the organocatalyst improved the addition yield to 48%. Unfortunately, increasing the quantity of the TMS protected diphenylprolinol to 75% or even an equimolar ratio did not show any yield improvement. In all cases, the reaction did not go to completion.

Further reduction of the β-amino aldehyde **8** with sodium borohydride provided alcohol **12** in good yield and without the need for further purification (Scheme 5a). We attempted to cyclize this alcohol in a one-pot fashion using nonafluorobutanesulfonyl fluoride (NfF) and DBU. After treatement of alcohol with NfF (1.1 equiv) and DBU (3.2 equiv) in a mixture of CH_2Cl_2:pentane 1:2 at 0 °C for 1 h, we were able to observe partial conversion to the desired cyclic product. However, due to the high basicity of DBU the alcohol **12** underwent transesterification and the desired product **4** was obtained together with the byproduct **13** (1:1 ratio). We evaluated the use of pyridine as base or lowering the temperature to –15 °C, but the outcome of the reaction was not favorable and the isolated yield was never higher than 38%.

We next examined the stepwise preparation of cyclic ester **4.** Mesylation of alcohol **12** smoothly afforded compound **9** in complete conversion. Treatment of the crude mesylated compound **9** with TBAF (1.5 equiv, 1 M in THF) at low substrate concentration (0.05 M) at 4 °C for 2 h furnished cyclic ester **4** as a single product (Scheme 5b). Hydrolysis of the ethyl ester afforded the *(S)-N*-Boc-5-Opr building block **1** in 96:4–98:2 er and good overall yield (60% over four steps). The *(S)-N*-Boc-5-Opr **1** was derivatized with *p*-bromoaniline, and the er of the



Scheme 5. a) Design of a new strategy towards the synthesis of (*S*)-*N*-Boc-5-oxaproline. b) Synthesis of (*S*)-*N*-Boc-5-oxaproline from alcohol **12.**

newly formed product was investigated (see experimental part). With a practical method to prepare *(S)-N*-Boc-5-Opr **1** in hand, we set out to explore the scalability of this route and we established a new route for the preparation of more than 5 g of *(S)-N*-Boc-5-Opr with a good overall yield. Ultimately, a research partner prepared 1 kg of desired oxaproline using our new route.

The (*S*)-*N*-Boc-5-Opr could also serve as a starting point for a variety of different protected building blocks for sequential KAHA ligations.[22,23]

3. Conclusions

We have developed a scalable and practical synthesis of the enantioenriched (*S*)-5-oxaproline building block using an improved enantioselective organomediated reaction. The key aldehyde intermediate **8** is also a valuable material for the preparation of novel monomers for KAHA ligation.

4. Experimental

^{1}H an^{13}C NMR spectra were recorded on Bruker DRX400 and Bruker AVIII400 spectrometers using CDCl3 as solvent. Chemical shift (δ) are reported in ppm with the solvent resonance as the internal standard relative to chloroform (δ 7.26 ppm for ^{1}H and 77.0 ppm for ^{13}C).

HRMS data were recorded by the mass spectrometry service of the laboratory of Organic Chemistry at ETH Zurich either with a Bruker maXis instrument equipped with an ESI source and a Qq-TOF detector.

TLC analysis was performed using pre-coated glass plate (Merck, silica gel 60 F254) and visualized by ultraviolet at 254 nm, by staining with potassium permanganate or cerium ammonium molybdate. Products were purified via column chromatography using silica gel type F60 (230–400 mesh) using a forced flow air at 0.5–1.0 bar.

4.1. Synthesis of starting materials

*4.1.1. (S)-2-(Diphenyl((trimethylsilyl)oxy)methyl)pyrrolidine (**7**)*

To *(S)*-diphenylprolinol (4.00 g, 15.8 mmol, 1.00 equiv) in CH_2Cl_2 (40 mL) was added imidazole (3.22 g, 47.4 mmol, 3.00 equiv) at 0 ºC. TMSCl (5.00 mL, 39.5 mmol, 2.50 equiv) was added dropwise and the reaction was stirred for 12 h at rt. MTBE (100 mL) was added to the reaction and the mixture was filtered. The organic phase was washed with H_2O (50 mL) and saturated aqueous NaCl (2 x 50 mL), dried over $MgSO_4$, filtered and concentrated under reduced pressure to a colorless oil **11** (5.00 g, 15.3 mmol, 97%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.54 – 7.46 (m, 2H), 7.42 – 7.36 (m, 2H), 7.35 – 7.20 (m, 6H), 4.07 (t, *J* = 7.4 Hz, 1H), 2.98 – 2.75 (m, 2H), 1.84 – 1.72 (m, 1H), 1.68 – 1.55 (m, 3H), 1.48 – 1.37 (m, 1H), -0.06 (s, 9H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ 146.83, 145.78, 128.44, 127.61, 127.57, 127.53, 126.90, 126.73, 83.17, 65.42, 47.16, 27.51, 25.06, 2.20 ppm. HR-MS (ESI): calculated for ($C_{20}H_{28}NOSi$) $[M+H]^+$: 326.1935, found: 326.1937.

*4.1.2. tert-Butyl (tert-butyldimethylsilyl)oxycarbamate (**5**)*

A 500 mL round-bottom flask charged with *tert*-butyl hydroxycarbamate (10 g, 75 mmol, 1.0 equiv) in CH_2Cl_2 (150 mL) and Et_3N (11.1 mL, 82.5 mmol, 1.10 equiv) was cooled to 0 ºC, and TBSCl (11.2 g, 75.0 mmol, 1.00 equiv) in CH_2Cl_2 (100 mL) was added. The reaction was allowed to warm to ambient temperature and stirred for 12 h. Upon completion, the reaction was diluted with H_2O, the organic layer washed with H_2O, saturated aqueous NaCl, dried over $MgSO_4$ and concentrated under reduced pressure to afford **5** (18.5 g, 75.0 mmol, quant) as a low melting solid that was used in the next step without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.67 (s, 1H), 1.48 (s, 9H), 0.95 (s, 9H), 0.162 (s, 6H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ –5.1, 18.7, 26.5, 28.8, 82.2, 158.5 ppm. HR-MS (ESI): calculated for ($C_{11}H_{25}NO_3Si$) $[M+Na]^+$: 270.1496, found 270.1497. IR (thin film): υ = 3292, 2960, 1752, 1693, 1473, 1463, 1392, 1367, 1251, 1171 cm^{-1}.

*4.1.3. Ethyl trans-4-oxo-2-butenoate (**6**)*

Ethyl *trans*-4-oxo-2-butenoate **6** was obtained from ABCR-Chemicals (96%) and purified by distillation. Ethyl *trans*-4-oxo-2-butenoate (20 mL) was placed in a 100 mL flask with a magnetic stirbar. The flask was equipped with a Vigreux containing a thermometer on the top and a water condenser on the side to collect the distillate. The pure fractions distilled at 35 °C at 8.10^{-2} mbars and the product was obtained with an isolated yield of 85%.

4.2. Synthesis of (S)-N-Boc-5-oxaproline

*4.2.1. (S)-Ethyl 2-((tert-Butoxycarbonyl)((tert-butyldimethylsilyl)oxy)amino)-4-oxobutanoate (**8**)*

To a solution of (*S*)-(–)-α,α-Diphenyl-2-pyrrolidinemethanol trimethylsilyl ether **7** (19.0 g, 58.6 mmol, 0.50 equiv) in $CHCl_3$ (130 mL) was added dropwise ethyl (*2E*)-4-oxo-2-butenoate **6** (14.1 mL, 117 mmol, 1.00 equiv) at 4 ºC. *tert*-Butyl (*tert*-butyldimethylsilyl)oxycarbamate **5** (34.8 g, 141 mmol, 1.20 equiv) was dissolved in $CHCl_3$ (70 mL) and added dropwise over 1 h. The reaction was stirred for 16 h at rt. TLC was taken in 25% EtOAc in hexanes using $KMnO_4$ as stain (product **8** has an Rf = 0.6 the starting material **5** has an Rf = 0.7 and the starting material aldehyde **6** has an Rf = 0.5). The reaction mixture was concentrated under reduced pressure. The crude compound was purified by column chromatography (gradient of 5–15% EtOAc in hexanes) to give **8** (21.2 g, 97:3 er, 56.1 mmol, 48% yield) as a pale yellow oil. HPLC: Chiralcel IA, hexanes:isopropyl alcohol 97:3, 20 °C, 30 µL, 0.8 mL/min, 210 nm, t_R = 6.2 min (major), t_R = 6.8 min (minor). ^{1}H NMR (400 MHz, CDCl3) δ 9.83 (t, *J* = 1.1 Hz, 1H), 4.91 (dd, *J* = 8.1, 5.3 Hz, 1H), 4.32 – 4.09 (m, 2H), 3.24 (ddd, *J* = 17.5, 8.1, 1.3 Hz, 1H), 2.80 (ddd, *J* = 17.6, 5.3, 0.9 Hz, 1H), 1.49 (s, 9H), 1.36 – 1.19 (m, 3H), 0.93 (s, 9H), 0.17 (d, *J* = 7.0 Hz, 6H) ppm. ^{13}C NMR (101 MHz, CDCl3) δ 198.70, 169.05, 157.90, 82.56, 61.65, 60.52, 42.76, 28.17, 28.14, 25.88, 25.86, 17.94, 14.16, -4.71, -4.79 ppm. HR-MS (ESI): calculated for ($C_{17}H_{33}NNaO_6Si$) $[M+Na]^+$: 398.1969, found: 398.1968. $[\alpha]_D^{26.4}$ = – 23.23 (c = 2.25, CH_2Cl_2). IR (thin film): υ = 3381, 2979, 2931, 2858, 1729, 1462, 1369, 1302, 1164, 1042, 1014, 835, 785 cm^{-1}.

*4.2.2. Ethyl N-(tert-Butoxycarbonyl)-N-((tert-butyldimethylsilyl)oxy)-L-homoserinate (**12**)*

The aldehyde **8** (15.0 g, 40.0 mmol, 1.0 equiv, 97:3 er) was dissolved in MeOH (200 mL, concentration of substrate 0.20 M) and sodium borohydride (3.02 g, 79.8 mmol, 2.0 equiv) was added in ten portions (~300 mg every 3 min) at –20 ºC. After complete addition, the reaction was stirred for 45 min in the CH_3CN-dry ice bath at -20 ºC, after which the reaction was allowed to warm to 0 ºC. The reaction was monitored by TLC in 25% EtOAc in hexanes using ninhydrin for stain (product **12** has Rf = 0.33 and stains violet and starting material **8** has Rf = 0.65 and stains yellow). Ice-water was added to the solution with vigorous stirring and the solution was stirred for 10 min at 0 ºC. To this mixture EtOAc and H_2O were added and the aqueous solution was poured into a 2-L separatory funnel. The aqueous layer was extracted with EtOAc (3 x 150 mL). The combined

organic layers were washed with saturated aqueous NH_4Cl (100 mL) and saturated aqueous NaCl (100 mL). The organic layer was dried over Na_2SO_4, filtered and concentrated under reduced pressure to a pale yellow oil **12** (14.8 g, 39.2 mmol, 98.2%) that was used without further purification. ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.47 (dd, *J* = 8.8, 5.8 Hz, 1H), 4.22 (dd, *J* = 7.1, 5.1 Hz, 2H), 3.77 (t, *J* = 5.7 Hz, 2H), 2.50–2.00 (m, 2H), 1.50 (s, 9H), 1.29 (d, *J* = 7.2 Hz, 3H), 0.95 (s, 9H), 0.21 (d, *J* = 3.1 Hz, 6H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$): δ 172.77, 157.40, 83.09, 65.67, 60.86, 60.39, 50.88, 28.20, 28.13, 26.92, 25.90, 24.95, 21.05, 17.98, 14.20, -4.87, -4.90 ppm.

*4.2.3. Ethyl N-(tert-Butoxycarbonyl)-N-((tert-butyldimethylsilyl)oxy)-O-(methylsulfonyl)-L-homoserinate (**9**)*

The oil **12** (14.8 g, 39.2 mmol, 1.0 equiv) was diluted with CH_2Cl_2 (195 mL) and Et_3N (16.6 mL, 119 mmol, 3.0 equiv) was added afterwards at 4 ºC. Methanesulfonyl chloride (7.40 mL, 75.7 mmol, 1.93 equiv) was added dropwise. The reaction mixture was stirred for 15 min at 4 ºC after which the ice-water bath was removed and the reaction was stirred at rt for 1 h. The reaction was monitored by TLC in 40% EtOAc in hexanes using ninhydrin stain (product **9** has Rf = 0.65 and starting material **12** has Rf = 0.57). Saturated aqueous NH_4Cl (150 mL) was added to the reaction and the mixture was poured into a 2-L separatory funnel. The combined organic layers were washed with saturated aqueous NH_4Cl (200 mL), saturated aqueous $NaHCO_3$ (200 mL) and saturated aqueous NaCl (200 mL). The organic layer was dried over Na_2SO_4, filtered and concentrated under reduced pressure. The resulting dark-orange oil containing ethyl *N*-(tert-butoxycarbonyl)-*N*-((tert-butyldimethylsilyl)oxy)-*O*-(methylsulfonyl)-L-homoserinate **9** was used in the next step with no further purification (17.6 g, 38.6 mmol, 98.1% crude yield, 83% purity by NMR). ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.75 (dd, *J* = 8.8, 5.8 Hz, 1H), 4.58 (dd, *J* = 7.1, 5.1 Hz, 2H), 4.40 (m, 2H), 3.21 (s, 3H), 2.63–2.46 (m, 2H), 1.68 (s, 9H), 1.48 (d, *J* = 7.2 Hz, 3H), 1.21 (s, 9H), 0.38 (d, *J* = 3.1 Hz, 6H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$): δ 177.71, 162.29, 87.95, 70.59, 65.78, 33.02, 30.80, 29.85, 22.87, 0.03, 0.00 ppm.

*4.2.4. (S)-2-(tert-Butyl) 3-ethyl (S)-isoxazolidine-2,3-dicarboxylate (**4**)*

The compound **9** (17.6 g, 38.6 mmol, 1.0 equiv) was diluted with THF (775 mL) tetrabutylammonium fluoride (1 M in THF, 58.0 mL, 58.0 mmol, 1.50 equiv) was added drpwise at 4 ºC. The reaction mixture was stirred for 1 h at 4 ºC. The reaction was monitored by TLC in 40% EtOAc in hexanes using ninhydrin as stain (product **4** has Rf = 0.45 and stains yellow and starting material **9** has Rf = 0.65 and stains brown). Saturated aqueous $NaHCO_3$ (120 mL) was added to the reaction and stirred for 10 min. The aqueous solution was diluted with Et_2O (300 mL) and the mixture was poured into a 2-L separatory funnel. The aqueous layer was separated and washed with Et_2O (3 x 150 mL). The combined organic layers were washed with saturated aqueous $NaHCO_3$ (150 mL) and saturated aqueous NaCl (150 mL). The organic layer was dried over Na_2SO_4, filtered and concentrated under reduced pressure. The resulting brown oil containing (*S*)-2-*tert*-butyl 3-ethyl isoxazolidine-2,3-dicarboxylate was purified by column chromatography (isocratic 20% EtOAc in hexanes) to furnish **4** (6.34 g, 25.8 mmol, 65.0% over 3 steps) as a clear yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$)**:** δ 4.66 (ddd, J = 4.8 Hz, 9.0 Hz, 9.4 Hz, 9.0 Hz), 4.21 (q, J = 7.1 Hz, 2H), 4.15–4.08 (m, 1H), 3.81 (ddd, J = 8.0 Hz, 8.0 Hz, 8.0 Hz, 1H), 2.66–2.55 (m, 1H), 2.49–2.38 (m, 1H), 1.48 (s, 9H), 1.28 (t, J = 7.1 Hz, 3H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$)**:** δ 170.6, 155.8, 82.6, 68.5, 61.8, 59.6, 33.2, 28.3, 14.4 ppm.

*4.2.5. (S)-2-(tert-Butoxycarbonyl)isoxazolidine-3-carboxylic acid (**1**)*

The oil **4** (6.34 g, 25.8 mmol, 1.0 equiv) was diluted with THF (105 mL) and was added a chilled solution of aqueous 1 M LiOH (103 mL, 103 mmol, 4.0 equiv) at 4 ºC. The reaction mixture was stirred for 1.5 h at rt. The reaction was monitored by TLC in 5% MeOH in CH_2Cl_2 using ninhydrin for staining. $CHCl_3$ (250 mL) was added and the solution was acidified to pH 3 with an aqueous solution of 2 M $KHSO_4$ (100 mL). The mixture was poured into a 1-L separatory funnel. The aqueous layer was separated and washed with $CHCl_3$ (5 x 100 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure to a clear yellow oil **1** that solidified upon standing in the refrigerator (5.39 g, 24.8 mmol, 96.0% yield, 62.4% overall yield, 97:3 er). ^{1}H NMR (400 MHz, $CDCl_3$)**:** δ 7.43 (s, 1H), 4.75–4.70 (m, 1H), 4.73 (dd, J = 5.2 Hz, 9.3 Hz, 1H), 3.85 (ddd, J = 8.0 Hz, 8.0 Hz, 8.0 Hz, 1H), 2.72–2.50 (m, 2H), 1.50 (s, 9H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$)**:** δ 174.5, 156.0, 83.2, 68.4, 59.4, 32.7, 28.0 ppm.

4.3. Determination of the enantiomeric ratio of (S)-N-Boc-5-oxaproline

*4.3.1. tert-Butyl (S)-3-((4-bromophenyl)carbamoyl)isoxazolidine-2-carboxylate (**S1**)*

To **1** (1.0 mg, 4.6 μmol, 1.0 equiv) in DMF (19 μL), *N,N'*-diisopropylcarbodiimide (1.4 μL, 9.2 μmol, 2.0 equiv), hydroxybenzotriazole (1.2 mg, 9.2 μmol, 2.0 equiv) were added and the mixture stirred for 10 min at rt. To this, *p*-bromoaniline (1.6 mg, 9.2 μmol, 2.0 equiv) was added and the reaction stirred at rt for 3 h. The mixture was diluted with CH_2Cl_2 (3 mL) and washed with H_2O (3 mL), saturated aqueous NaCl (3 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was diluted with a mixture of 9:1 hexanes:isopropyl alcohol (250 μL) and separated by preparative achiral HPLC (normal phase, 9:1 hexanes:isopropyl alcohol) on an Alltima column to give amide derivate **S1** (1.1 mg, 2.9 μmol, 97:3 er, 64% yield). HPLC: Chiralcel IA, hexanes/isopropyl alcohol 8:2, 20 °C, 30 μL, 1.0 mL/min, 210 nm, tR = 8.3 min (minor), tR =15.95 min (major). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.51 (s, 1H), 7.62 – 7.40 (m, 3H), 4.93 – 4.74 (m, 1H), 4.14 (dddd, *J* = 8.4, 7.4, 4.4, 0.8 Hz, 1H), 3.93 – 3.67 (m, 1H), 2.66 (ddd, *J* = 8.8, 4.3, 3.4 Hz, 1H), 1.54 (s, 9H) ppm. ^{13}C NMR (151 MHz, $CDCl_3$) δ 168.43, 157.63, 136.31, 131.95, 121.41, 117.22, 84.05, 69.38, 62.71, 32.45, 28.08 ppm.

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