DOI: 10.1002/ejoc.200701016

Synthesis of a Naturally Occurring Diene-Containing Amino Acid and Its Glutamyl Dipeptide via *N*-Acyliminium Ion Chemistry

Martin Berkheij,[a] Jan Dijkink,[a] Olivier R. P. David,[b] Theo Sonke,[c] Denis R. IJzendoorn,[d] Richard H. Blaauw,[d] Jan H. van Maarseveen,[a] Hans E. Schoemaker,[a,c] and Henk Hiemstra*[a]

Keywords: Natural products / Dienes / Enzymatic resolutions / Peptides / Allylsilanes

The syntheses of the naturally occurring unusual unsaturated amino acid (*S*)-2-amino-3-methylene-4-pentenoic acid and the corresponding γ-glutamyl dipeptide are described. Key steps are an *N*-acyliminium ion addition using allenylmethylsilane as nucleophile and an enzymatic resolution mediated by the L-aminopeptidase from *Pseudomonas putida*.

Introduction

The leaves of the ornamental shrub *Philadelphus coronarius* (Hydrangeaceae) contain a number of unusual amino acids and glutamylpeptides.[1] Among these natural products are the dienic α-amino acid **1a** and its γ-glutamylpeptide **1b** (Figure 1). The dipeptide **1b** appeared quite unstable during the isolation, while the amino acid **1a** could not be isolated at all. Nevertheless, the authors reported the harvest of 150 mg of **1b** from 4400 g of leaves. The glutamyl part of dipeptide **1b** appeared to have the natural (*S*)-configuration, but little further information about the properties of these interesting diene amino acids is available.

The α-amino acid **1a** belongs to a fairly large group of unusual unsaturated linear, branched and cyclic α-amino acids, which were isolated in recent decades from several different biological sources.[1–6] A limited selection of these interesting molecules is depicted in Figure 1. Some of these amino acids also occur as their corresponding γ-glutamyl dipeptides, like **1b** and **4b**. Although the exact biological relevance of these amino acids is unknown they are believed to play a role in the defence against predators, either as toxin or as an intermediary metabolite.[7] For example, hypoglycin A and B (**4a** and **4b** in Figure 1)[3,4,6] are known to cause Jamaican vomiting sickness and ultimately death.[7]

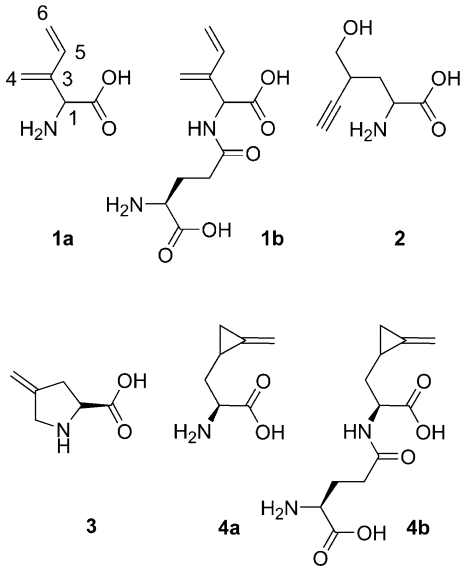


Figure 1. Natural unsaturated α-amino acids.

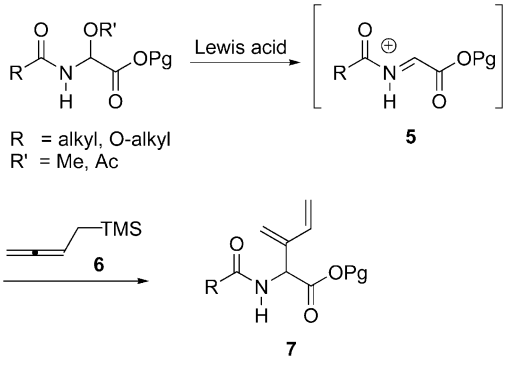
The salient feature of **1a**,**b** is the 1,3-diene function which is expected to make these molecules quite sensitive to acidic and basic conditions. The structural assignment of **1a** and **1b** is based on scarce spectroscopic data and on the amount of hydrogen taken up in a hydrogenation reaction.

Amino acid **1a** can be regarded as a tetradehydro derivative of isoleucine (tdIle). The diene moiety offers a unique site for further derivatization, as was already described for the homologous diene amino acid.[8,9] For a synthesis of **1a** one cannot readily rely on the functionalization of glycine anion equivalents. Instead, a synthesis proceeding via a cat-

[a] Van't Hoff Institute for Molecular Sciences, Faculty of Science, University of Amsterdam,
Nieuwe Achtergracht 129, 1018 WS Amsterdam, The Netherlands
Fax: +31-20-5255670
E-mail: hiemstra@science.uva.nl
[b] Institute Lavoisier de Versailles, UMR 8180, Université de Versailles,
Avenue des Etats-Unis, 78035 Versailles Cedex, France
[c] DSM Pharmaceutical Products – Advanced Synthesis, Catalysis & Development,
P. O. Box 18, 6160 MD Geleen, The Netherlands
[d] Chiralix BV,
P. O. Box 31070, 6503 CB Nijmegen, The Netherlands

ionic intermediate, i.e. an *N*-acyliminium ion, seems a more logical option. In view of our expertise in this area and our desire to investigate the synthesis and properties of diene amino acids of type **1a** we undertook this investigation which is described herein.

N-Acyliminium ion chemistry is a powerful method for the formation of C–C bonds,[10,11] and has been intensively studied in our group for many years.[12,13] We envisioned that these tetradehydro-isoleucine (tdIle) derivatives could be prepared using a method developed earlier in our group, using allenylmethylsilane **6** as a nucleophile in a reaction with a glycine derived *N*-acyliminium ion **5** (Scheme 1).[14] In this article we report the first total syntheses of **1a** and **1b** employing this strategy. In addition we detail the enzymatic resolution of the diene amino acid **1a**.



Scheme 1.

Results and Discussion

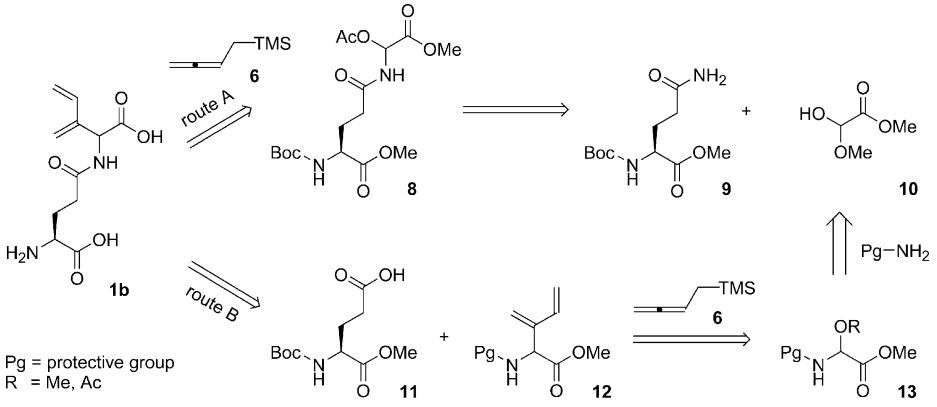
The retrosynthetic approach towards pseudodipeptide **1b** is illustrated in Scheme 2. We distinguished two options for its synthesis, namely via an *N*-acyliminium ion reaction of allenylmethylsilane **6** with γ-glutamyl dipeptide **8** (route A, Scheme 2) or by carrying out a peptide coupling between the suitably protected glutamic acid **11** and diene **12** (route B). Dipeptide **8** was expected to arise from condensation of suitably protected glutamine **9** with hemiacetal **10**. Condensation of **10** with a simple carbamate or amide of choice should lead to N,O-acetal **13**, the precursor for the preparation of the protected diene amino acid **12**.

First, we investigated the racemic synthesis of α-amino acid **1a**. Protected α-hydroxyglycine **14** was obtained by condensation of methyl carbamate with methyl glyoxylate methyl hemiacetal **10**[15] under Dean–Stark conditions in cyclohexane (Scheme 3). Under these conditions the product precipitated from the reaction mixture. Treatment of **14** with acidic methanol gave the more stable *N*-acyliminium ion precursor **15** in 63% yield over two steps (Scheme 3). Coupling of allene **6**[14,16] with **15** was performed in acetonitrile at 0 °C, using $BF_3{\cdot}OEt_2$ as the Lewis acid providing the desired α-diene amino acid **16** in 87% yield.

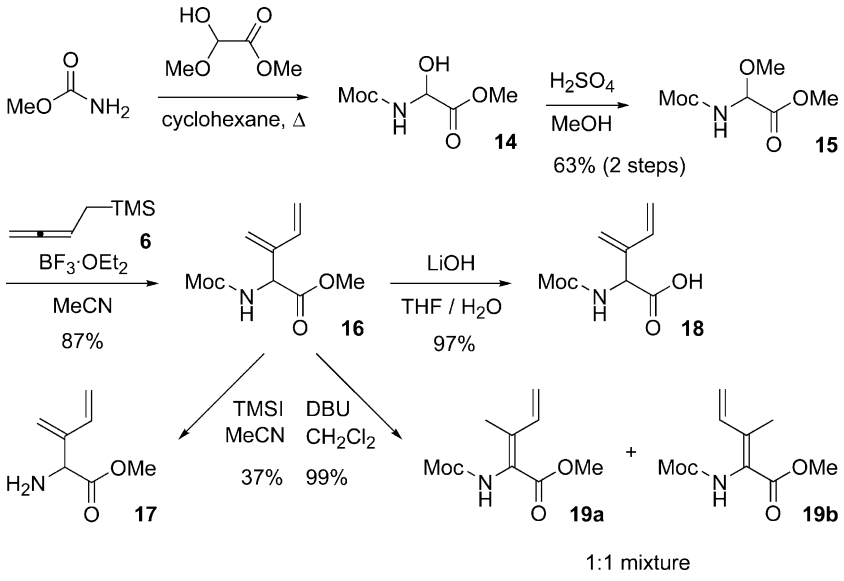
Next, the consecutive liberation of the acid and the amine group was studied. Saponification of the methyl ester using LiOH in aqueous THF gave free acid **18** in 97% yield. Gratifyingly, no double bond isomerization was observed under these conditions. Curious to know which circumstances would lead to isomerization, we added a catalytic amount (ca. 15%) of DBU to a solution of **16** in dichloromethane. In this way the expected conjugated diene amino acids **19a** and **19b** were obtained as a 1:1 mixture in quantitative yield. Removal of the methoxycarbonyl (Moc) group in **16** by using trimethylsilyl iodide (TMSI) in acetonitrile provided free amine **17**, albeit in a disappointing 37% yield. Possibly, simultaneous with the nitrogen liberation, the ester is also cleaved via a similar mechanism.[17] This troublesome Moc-removal prompted us to follow a different protective group strategy.

We therefore turned our focus to a Boc-protection strategy, because this would allow an orthogonal deprotection of the amine and the acid moieties. So we prepared Boc-α-hydroxyglycine methyl ester **20** (79%, Scheme 4) using the same strategy as used for the synthesis of **14**. However, conversion of **20** into its α-methoxy derivative using a catalytic amount of sulfuric acid in methanol resulted in partial loss of the Boc protective group.

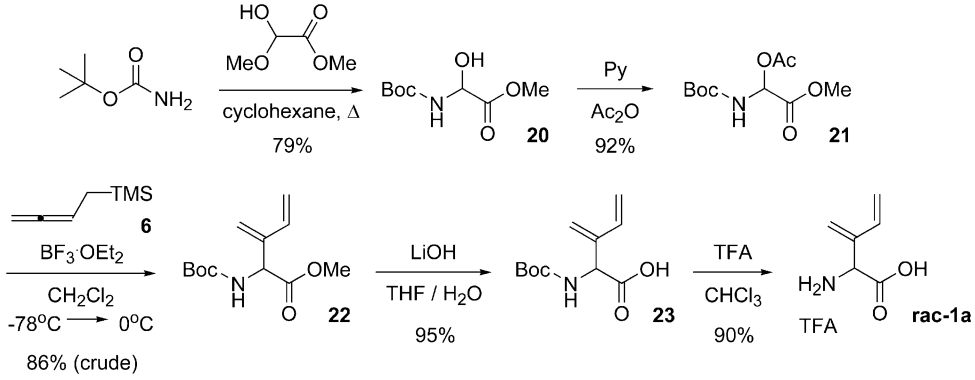
Therefore, we decided to use the acetoxy group as an improved leaving group. The acetate was introduced by stirring of **20** in acetic anhydride in the presence of a catalytic amount of pyridine, providing **21** in 92% yield. Initial attempts to couple **21** with allenylmethylsilane **6** under the same conditions as used previously for the synthesis of **16**, led to a substantial $BF_3{\cdot}OEt_2$ induced loss of the Boc group



Scheme 2.



Scheme 3.

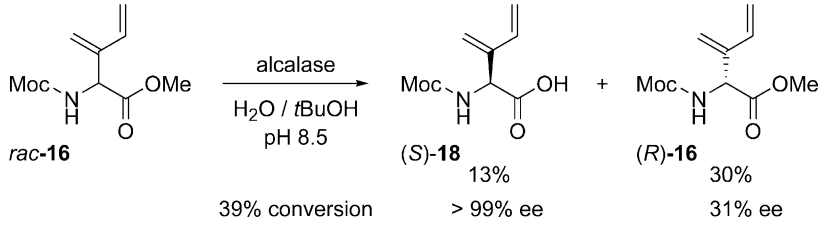


Scheme 4.

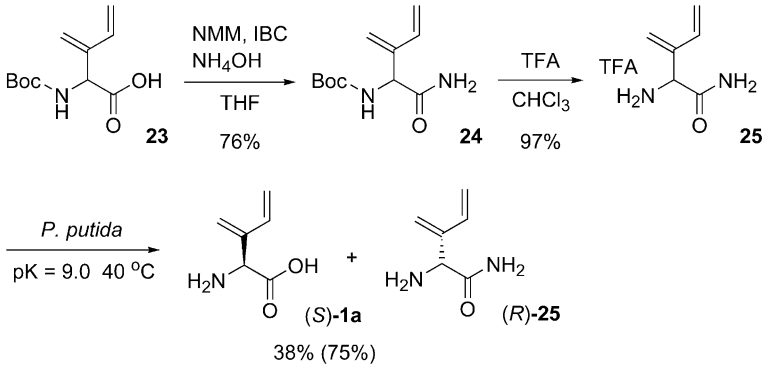
from both **21** and **22**. Therefore, the temperature was lowered to –78 °C and dichloromethane was used as the solvent. At this low temperature no reaction was observed and the reaction mixture was warmed-up slowly while closely monitoring the reaction by TLC. These reaction conditions provided the desired **22** in 86% yield as a crude product containing some impurities as judged from TLC and NMR spectroscopy. Attempts to purify **22** by column chromatography resulted in considerable loss of material, most probably due to polymerization.[18]

Thus, crude **22**, containing only minor amounts of impurities, was used without further purification in the next step (Scheme 4). After saponification of the methyl ester using LiOH (**23**, 95%) and subsequent Boc-removal (90%), racemic 2-amino-3-methylene-4-pentenoic acid (**1a**) was isolated. Using this sequence, natural product **1a** was obtained as a racemic TFA-salt in 53% yield over 5 steps (Scheme 4). The ^{1}H NMR spectrum and TLC-properties were in agreement with those reported in the literature.[1] With the racemic product in hand we focussed on the synthesis of the pure enantiomers.

In order to gain access to enantiopure **1a**, we carried out an enzymatic resolution[19] on **16**, using the lipase Alcalase® (Novozymes Alcalase 2.5 L DX, Scheme 5).[20,21] Prelimi-



Scheme 5.



Scheme 6.

nary experiments showed that (*S*)-**16** was selectively converted into its free acid (*S*)-**18** whereas the (*R*)-ester **16** remained untouched as monitored by chiral HPLC[22] (Scheme 5). When the reaction was stopped at 39% conversion, (*S*)-**18** could be isolated in 13% yield with >99% enantiomeric excess (*ee*). The (*R*)-enriched methyl ester **16** was recovered in 30% yield and 31% *ee*. These small-scale experiments also showed that after 45% conversion the *ee* of acid **18** dropped, indicating that the enzyme is not completely enantioselective.

Therefore, we turned our attention to the L-aminopeptidase PepA from *Pseudomonas putida*, which is known to hydrolyze α-amino amides with high (*S*)-selectivity.[23] As it requires amino amides as substrates, acid **23** was converted into its corresponding amide **24** via the mixed anhydride method (Scheme 6) using, successively, *N*-methylmorpholine/isobutyl chloroformate and ammonia[24] in 76% yield. Boc removal provided amino amide **25** (97%) as the substrate for the enzymatic resolution. In a small scale experiment the L-aminopeptidase from *P. putida* appeared to be completely selective, as even 15 h after reaching 50% conversion, the *ee* of the product remained >99% (Figure 2). The actual aminopeptidase mediated resolution was performed using a known procedure,[23] but separation of the product and starting material via the Schiff base of benzaldehyde proved to be tedious. The (*R*)-amino amide (*R*)-**25** (partly) racemized in the Schiff-base stage, but fortunately (*S*)-amino acid **1a** could be isolated in a satisfying 38% yield (75% based on one enantiomer). Thus, the (*S*)-enantiomer of the naturally occurring 2-amino-3-methylene-4-pentenoic acid (*S*)-**1a** has been synthesized in 17% yield over 7 steps. With the enantiopure diene amino acid in hand, we aimed for the synthesis of the natural glutamyl dipeptide **1b**.

Synthesis of the iminium ion precursor **27** (Scheme 7) to allow for the *N*-acyliminium ion reaction in the dipeptide (route A, Scheme 2) was straightforward. Thus, condensation of methyl glyoxylate methyl hemiacetal **10** with *N*-Boc-(*S*)-glutamine methyl ester **9** (readily available from *N*-Boc-glutamine by esterification with trimethylsilyldiazomethane) gave suitably protected γ-glutamyl hydroxyglycine **26** (58%), which was readily converted into the acetoxy derivative **27** in 97% yield. For the condensation, cyclohexane was

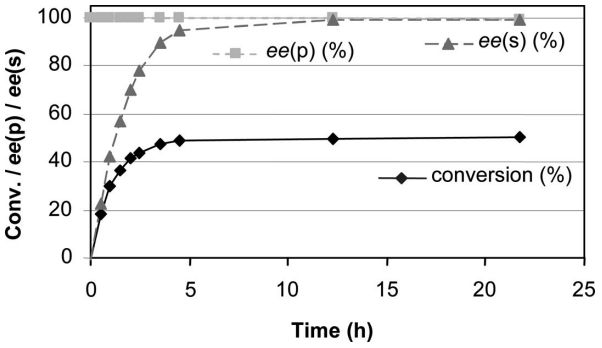
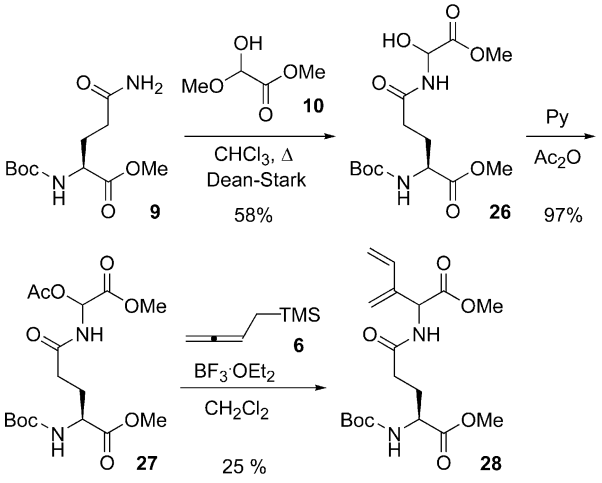


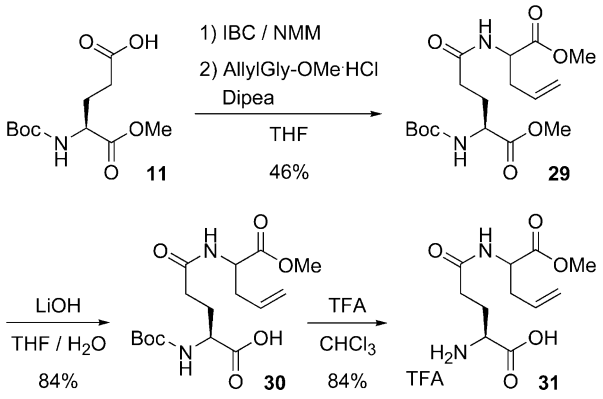
Figure 2. Resolution of **25** by *P. putida* L-aminopeptidase.

replaced by $CHCl_3$ for solubility reasons. However, when the *N*-acyliminium ion reaction was performed under the same conditions as used in the synthesis of amino acid **22**, dipeptide **28** was isolated as a ca. 1:1 mixture of diastereomers in a disappointing 25% yield. The reaction showed slow conversion, but raising the temperature resulted in partial Boc deprotection and the use of other Lewis acids did not improve the yield either. Furthermore, **28** was quite unstable. Aiming at a diastereoselective synthesis we then decided to synthesize **1b** by using the dipeptide coupling approach (route B, Scheme 2).



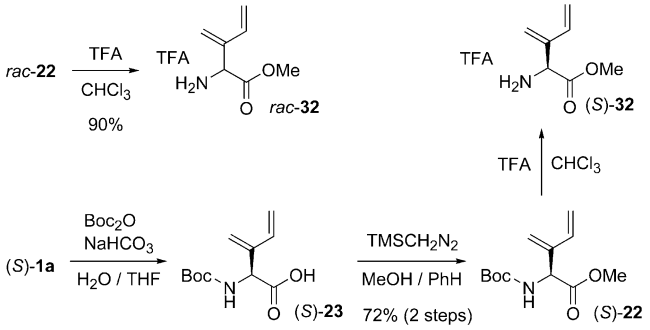
Scheme 7.

Synthesis of the dipeptide via a peptide coupling approach was first tested on racemic allylglycine (Scheme 8) using the mixed anhydride method which was also used for the synthesis of **24** (Scheme 6). Commercially available allylglycine was first converted into its corresponding methyl ester using $SOCl_2$ in MeOH.[25] An aqueous solution of allylglycine methyl ester was added to the mixed anhydride of IBC and Boc-Glu-OMe (**11**) to yield the desired dipeptide **29** in 46% yield and an expected 1:1 ratio of diastereomers (Scheme 8). An EDC/HOBt mediated peptide coupling gave **29** in 39% yield. After saponification of **29** by LiOH to give di-acid **30** and TFA-mediated Boc removal (both 84%) γ-glutamyl dipeptide **31** was obtained in 32% yield over 5 steps.



Scheme 8.

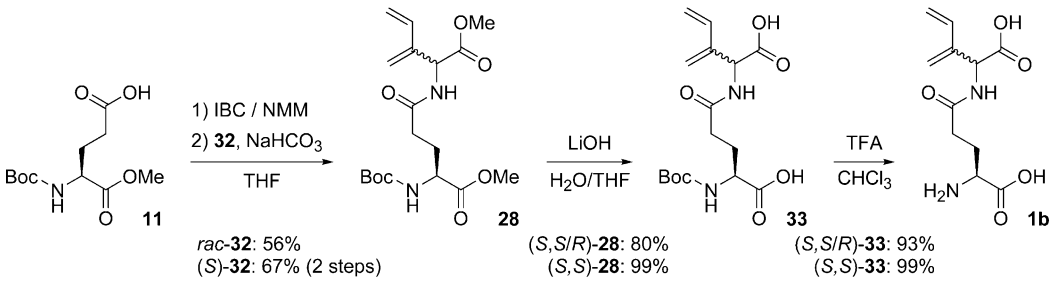
Now the stage was set for the synthesis of the naturally occurring dipeptide **1b**. As the derivatives of 1,3-butadiene-containing amino acids already proved to be more difficult to handle than, e.g., allylglycine (most probably caused by the C^3=C^4 bond, Figure 1), we decided to synthesize **1b** first by using racemic tdIle. TFA-mediated *N*-Boc deprotection of *rac*-**22** delivered our diene-methyl ester TFA salt **32** in 90% yield (Scheme 9). Subsequent coupling with Boc-Glu-OMe **11** via the mixed anhydride method using $NaHCO_3$ to liberate the amine of **32** gave the desired dipeptide **28** in 56% yield (Scheme 10). Other coupling conditions (NMM/IBC/DIPEA, HATU/DIPEA, EDC/HOBt/DIPEA or PyBop/DIPEA) all resulted in a lower yield of the dipeptide. Di-ester **28** was readily saponified to **33** in 80% yield, followed by Boc removal (93%) to give the desired natural product **1b** and its epimer as a 1:1 mixture of diastereomers in an overall yield of 23% over 7 steps.



Scheme 9.

The same sequence was repeated for de synthesis of (*S*,*S*)-γ-glutamyl-2-amino-3-methylene-4-pentenoic acid (*S*,*S*)-**1b**. Therefore, (*S*)-**32** was prepared via a protection/deprotection strategy (Scheme 9). First, a Boc-group was installed on the amine, followed by treatment of the resulting acid (*S*)-**23** with trimethylsilyldiazomethane to arrive at methyl ester (*S*)-**22** in 72% yield over two steps. Amine liberation using TFA/$CHCl_3$ (1:1) gave the desired TFA salt (*S*)-**32**. Now, subjection of (*S*)-**32** to the coupling conditions as used for the coupling of *rac*-**32** with **9** (Scheme 10) gave (*S*,*S*)-**28** in a satisfying 67% yield over two steps.

Finally, both saponification and Boc-removal of (*S*,*S*)-**28** proceeded smoothly, providing the desired dipeptide (*S*,*S*)-**1b** in 98% yield over two steps. The ^{1}H-NMR spectrum and TLC-properties were in agreement with those reported in literature, and therefore we could confirm the proposed structure of (*S*,*S*)-γ-glutamyl-2-amino-3-methylene-4-pentenoic acid **1b**. This naturally occurring 1,3-diene-containing γ-glutamyl dipeptide was synthesized in a stereoselective manner in 7.9% overall yield in 13 steps, starting from *tert*-butyl carbamate. Unfortunately, due to the limited availability of physical and spectroscopic data, and the absence of a sample of the original isolate, we were not able to deduce the absolute configuration of both the amino acid and dipeptide natural products.



Scheme 10.

Conclusions

In summary, *rac*-2-amino-3-methylene-4-pentenoic acid (tdIle) has been synthesized in 53% yield (5 steps) starting from *tert*-butyl carbamate using allenylmethylsilane as nucleophile in the addition to a glycine-derived *N*-acyliminium ion. We were able to confirm the structure of the compound containing an α-diene moiety as proposed by Campos et al. Both enantiomers of the amino acid were separated via an enzymatic kinetic resolution catalyzed by the L-aminopeptidase PepA from *P. putida*, and (*S*)-tdIle was isolated in 38% yield (75% based on one enantiomer) and > 99% *ee*. Separation and isolation of the (*S*)-amino acid and (*R*)-amino amide proved to be difficult and needs further optimalization. (*S*)-2-amino-3-methylene-4-pentenoic acid has been synthesized in 17% overall yield (7 steps).

Further, (*S*,*S*)-γ-glutamyl-2-amino-3-methylene-4-pentenoic acid was synthesized via a peptide coupling of (*S*)-2-amino-3-methylene-4-pentenoic methyl ester and Boc glutamic acid methyl ester in 7.9% (15.5% based on one enantiomer) over 13 steps (starting from *tert*-butyl carbamate). The diastereomeric dipeptide was prepared in 23% over 7 steps (starting from *tert*-butyl carbamate). *N*-acyliminium ion chemistry in the γ-glutamyl dipeptide is still troublesome, and is currently under further investigation.

Experimental Section

General Remarks: Unless stated otherwise, chemicals were purchased from commercial suppliers and used as such. Methyl glyoxylate methyl hemiacetal was kindly donated by DSM, Linz, Austria. Dichloromethane and acetonitrile were freshly distilled from calcium hydride. Tetrahydrofuran was freshly distilled from sodium using benzophenone as indicator. All reactions were carried out under an inert atmosphere of dry nitrogen, unless stated otherwise. Column chromatography was performed using Biosolve silica gel (230–460 mesh, 60 Å). R_f values were obtained by using Thin Layer Chromatography (TLC) on silica gel coated alumina sheets (Merck silica gel 60 F_{254}) with indicated solvents. Compounds were visualised by UV and/or exposure to $KMnO_4$, ninhydrine, or Cl_2/TDM (see below). Infrared spectra were recorded on a Bruker IFS 28 or a Shimadzu FTIR-8400S spectrophotometer. Nuclear magnetic resonance (NMR) data were obtained on a Bruker ARX 400, Bruker Avance 400 or a Varian Inova-500. Spectra are reported in units of ppm on the δ scale, relative to the solvent used. When a diastereomeric mixture of products was obtained, all relevant NMR signals for the mixture are given. Mass spectra were measured using a JEOL JMS SX/SX102A four-sector mass spectrometer, coupled to a JEOL MS-MP7000 data system. Melting points were determined on a Wagner & Munz Polytherm A and are uncorrected. Optical rotations were measured on a Perkin–Elmer 241 polarimeter.

Abbreviations: DBU = 1,8-diazabicyclo[5.4.0]undec-7-ene, DIPEA = diisopropylethylamine, EDC = 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide, HATU = *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate, HOBt = 1-hydroxybenzotriazole, IBC = isobutyl chloroformate, NMM = *N*-methylmorpholine, PyBop = (benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate, TDM = 4,4′-methylenebis-(*N*,*N*′-dimethylaniline).

Cl_2/TDM (for the visualisation of amides on TLC): Solution A: TDM 6.2 g/AcOH 25 mL/H_2O 125 mL. Solution B: KI 12.5 g/H_2O 250 mL. Solution C: ninhydrine 0.6 g/AcOH 20 mL/H_2O 180 mL. TDM solution: A:B:C = 30:50:0.75. Cl_2 source: $Ca(OCl)_2$. **Use:** Place TLC 1 min in jar filled with a 2-cm layer of $Ca(OCl)_2$. Remove TLC from jar and blow off excess Cl_2 with *cold* air. Then dip TLC in TDM solution and warm gently. Brown/green spots should appear on a slightly blue background. When plate is completely blue without spots: blow off Cl_2 longer or refresh TDM solution. When plate is covered with black spots: remove free Si particles from TDM solution via filtration.

Methyl 2-Hydroxy-2-(methoxycarbonylamino)acetate (14): A 500 mL flask containing a suspension of methyl carbamate (55 g, 0.732 mol) and methyl glyoxylate methyl hemiacetal (96.8 g, 80 mL, 0.806 mol) in cyclohexane (300 mL, HPLC grade) was equipped with a Dean Stark apparatus. The suspension was stirred vigorously at reflux for 15 h, followed by refrigeration until completely frozen. After the solvent had melted again it was decanted and the viscous crude product was carefully dried under high vacuum while heated in a water bath at 55 °C. After cooling down at room temperature, the crude hydroxyglycine **14** was obtained as a white solid and was used as such in the following step. (spectroscopic data identical to those reported in the literature).[26] ^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.88 (br. s, 1 H), 5.46 (br. d, J = 7.6 Hz, 1 H), 4.86 (br. d, J = 7.6 Hz, 1 H), 3.85 (s, 3 H), 3.73 (s, 3 H) ppm. IR (neat): $\tilde{\nu}$ = 3344, 2959, 1719, 1528, 1447, 1231, 1055 cm^{-1}. R_f(EtOAc) = 0.38.

Methyl 2-Methoxy-2-(methoxycarbonylamino)acetate (15): Crude **14** was dissolved in methanol (200 mL) and the solution was cooled to 0 °C. Trimethyl orthoformate (50 mL) was added followed by neat H_2SO_4 (0.2 mL). The mixture was warmed to room temperature and stirred for 15 h. Then a saturated aqueous NaHCO3 solution (10 mL) was added under vigorous stirring and left for 15 min when solid $NaHCO_3$ was added to "quench" the water. The mixture was filtered through a pad of neutral alumina (2 cm), which was washed with more methanol. The volatiles were removed in vacuo and while the viscous product was still warm, Et_2O (300 mL) was added leading to a clear solution. The solution was cooled to –18 °C and then left standing overnight, providing the impure methoxyglycine as a white solid. The compound was filtered, washed with diethyl ether and redissolved in CH_2Cl_2 (200 mL) and cooled to –18 °C for another night. The precipitate (**14**) was filtered and the filtrate was concentrated in vacuo to give **15** (82 g, 0.463 mol, 63% over two steps) as an off-white oil which crystallized upon standing as transparent needles. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.90 (br. s, 1 H), 5.33 (br. d, J = 7.6 Hz, 1 H), 3.82 (s, 3 H), 3.73 (s, 3 H), 3.46 (s, 3 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 168.07, 156.38, 80.62, 55.98, 52.75, 52.54 ppm. IR (neat): $\tilde{\nu}$ = 3356, 2956, 2835, 1736, 1524, 1441, 1342, 1220, 1057, 1000, 901 cm^{-1}. HRMS(FAB): calcd. for $[C_6H_{11}NO_5 + H]^+$: 178.0715, found: 178.0715; m.p. 33–35 °C, R_f(PE/EtOAc, 1:1) = 0.43.

Methyl 2-(Methoxycarbonylamino)-3-methylenepent-4-enoate (16): To a stirred solution of **15** (1.50 g, 8.47 mmol) in acetonitrile (45 mL) at 0 °C was added allenylmethyltrimethylsilane **6** (1.60 g, 12.7 mmol), followed by the drop wise addition of $BF_3{\cdot}OEt_2$ (1.60 mL, 12.7 mmol). The reaction was stirred at 0 °C for 3 h after which period the yellow solution was poured on a saturated aqueous $NaHCO_3$ solution and extracted with EtOAc (3×). The organic layer was washed with brine, dried with Na_2SO_4, filtered, and concentrated in vacuo to afford a yellow oil. Purification by column chromatography (PE/EtOAc, 3:1) afforded **16** (1.47 g, 7.37 mmol,

87%) as a colorless oil which solidified upon standing. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.32 (dd, J = 17.7, J = 11.2 Hz, 1 H), 5.44 (d, J = 17.7 Hz, 1 H), 5.41 (br. d, J = 8.8 Hz, 1 H), 5.30 (s, 1 H), 5.23 (s, 1 H), 5.19 (d, J = 11.2 Hz, 1 H), 5.12 (d, J = 8.8 Hz, 1 H), 3.75 (s, 3 H), 3.69 (s, 3 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 171.23, 155.97, 141.69, 134.93, 118.69, 115.69, 54.89, 52.53, 52.25 ppm. IR (neat): $\tilde{\nu}$ = 3333, 2954, 1719, 1523, 1206, 1060 cm^{-1}. HRMS(EI): calcd. for $[C_9H_{13}NO_4]^+$: 199.0845, found: 199.0832; R_f(PE/EtOAc, 1:1) = 0.61.

Methyl 2-Amino-3-methylenepent-4-enoate (17): In an oven dried 50 mL flask, **16** (0.253 g, 1.27 mmol) was dissolved in dry acetonitrile (25 mL). The solution was degassed (three vacuum-argon balloon cycles) and kept under an argon atmosphere. Then at room temperature, of iodotrimethylsilane (1 g, 725 μL, 5.09 mmol) was added in one portion, and the solution was stirred for 1 h. The mixture was poured on a 5% aqueous $NaHSO_4$ solution and extracted CH_2Cl_2 (3×) (be aware of emulsion formation). The aqueous fase was basified until pH 9 with solid K_2CO_3 and extracted with CH_2Cl_2 (5×). The combined organic layer was dried with Na_2SO_4 filtered and concentrated in vacuo at room temperature (to prevent diketopiperazine formation) to afford 56 mg of the crude amino-ester **17**, which was used as such in the next reaction. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.34 (dd, J = 17.7, 11.2 Hz, 1 H), 5.42 (d, J = 17.7 Hz, 1 H), 5.25 (s, 1 H), 5.21 (s, 1 H), 5.17 (d, J = 11.2 Hz, 1 H), 4.33 (s, 1 H), 3.74 (s, 3 H), 2.31 (br. s, 2 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 174.17, 145.07, 135.56, 116.79, 114.98, 55.89, 52.16 ppm. IR (neat): $\tilde{\nu}$ = 3373, 2953, 1739, 1595, 1436, 1201, 993, 909 cm^{-1}. R_f(EtOAc/MeOH, 2:1) = 0.17.

2-(Methoxycarbonylamino)-3-methylenepent-4-enoic Acid (18): To a stirred solution of **16** (0.100 g, 0.502 mmol) in THF (3 mL) at 0 °C was added drop wise a solution of LiOH (18 mg, 0.753 mmol) in H_2O (3 mL). The slight yellow mixture was stirred at 0 °C for 3 h when it was poured on 5% $KHSO_4$ and extracted with EtOAc (3×). The organic layer was washed with brine, dried with Na_2SO_4, filtered, and concentrated in vacuo to yield **18** (91 mg, 0.491 mmol, 98%) as a slight yellow oil. ^{1}H NMR (400 MHz, MeOD): δ = 6.42 (ddd, J = 0.4, 11.1, 17.6 Hz, 1 H), 5.44 (d, J = 17.7 Hz, 1 H), 5.34 (s, 1 H), 5.28 (s, 1 H), 5.19 (d, J = 11.1 Hz, 1 H), 5.04 (s, 1 H), 3.67 (s, 3 H) ppm. ^{13}C NMR (100 MHz, MeOD): δ = 172.62, 157.50, 142.40, 135.78, 117.64, 114.20, 54.73, 51.36 ppm. IR (neat): $\tilde{\nu}$ = 3314, 2960, 2360, 1716, 1521, 1061, 913 cm^{-1}. HRMS(FAB): calcd. for $[C_8H_{11}NO_4 + H]^+$: 186.0766, found: 186.0762; R_f(PE/EtOAc, 1:1) = 0.61.

Methyl 2-(Methoxycarbonylamino)-3-methylpenta-2,4-dienoate (19): To a solution of **16** (0.200 g, 1.00 mmol) in CH_2Cl_2 was added DBU (25 μL, 0.167 mmol) and the yellow mixture was stirred at room temperature for 69 h when it was poured into 5% $KHSO_4$ and extracted with EtOAc (3×). The organic layer was washed with brine, dried with Na_2SO_4, filtered, and concentrated in vacuo to yield **19** as a 1:1 mixture of (*E*,*Z*)-isomers in 99% yield (0.200 g, 1.00 mmol). ^{1}H NMR (400 MHz, MeOD): δ = 7.17 (dd, J = 11.0, 17.3 Hz, 0.5 H), 6.84 (dd, J = 10.9, 17.3 Hz, 0.5 H), 5.63 (d, J = 17.4 Hz, 0.5 H), 5.54 (d, J = 17.3 Hz, 0.5 H), 5.43 (d, J = 10.9 Hz, 0.5 H), 5.30 (d, J = 11.0 Hz, 0.5 H), 3.76 (s, 3 H), 3.70 (s, 3 H), 2.10 (s, 1.5 H), 1.94 (s, 1.5 H) ppm. ^{13}C NMR (100 MHz, MeOD): δ = 166.14, 165.43, 156.32, 155.95, 135.73, 134.86, 134.71, 133.36, 125.60, 125.06, 123.96, 118.35, 116.78, 51.42, 50.91, 50.83, 12.37, 12.22 ppm. IR (neat): $\tilde{\nu}$ = 2955, 1722, 1452, 1383, 1236, 1065, 921 cm^{-1}. HRMS(FAB): calcd. for $[C_9H_{13}NO_4 + H]^+$: 200.0923, found: 200.0926; R_f(PE/EtOAc, 1:1) = 0.50.

(*R*)-Methyl 2-(Methoxycarbonylamino)-3-methylenepent-4-enoate [(*R*)-16] and (*S*)- 2-(Methoxycarbonylamino)-3-methylenepent-4-enoic Acid [(*S*)-18]: To a stirred solution of **16** (0.109 g, 0.547 mmol) in H_2O/*t*BuOH (5 mL/0.3 mL) was added Alcalase® (Novozymes 2.5 l DX, PLN 04810; 50 μL). After stirring at room temperature for 24 h, 1 mL of enzyme was added and the mixture was heated at 37 °C for 6.5 h when aqueous HCl (10 mL, 1 N) was added. The mixture was extracted with EtOAc (3×) and the organic layer was washed with brine, dried with Na_2SO_4, filtered, and concentrated in vacuo. The crude brownish oil was purified by column chromatography (PE/EtOAc/AcOH, 2:1:0.01) to yield (*R*)-**16** in the first fraction as a colorless oil (24 mg, 0.121 mmol) in 22%. In the second fraction, (*S*)-**18** was isolated as a colorless oil (19 mg, 0.103 mmol) in 19%.

Data for (*R*)-16: Identical to *rac*-**16**. $[\alpha]_D^{19}$ = –39.0 (c = 0.4, MeOH)

Data for (*S*)-18: Identical to *rac*-**18**; *ee* 99% (HPLC), $[\alpha]_D^{19}$ = +58.7 (c = 0.3, MeOH).

Methyl 2-(*tert*-Butoxycarbonylamino)-2-hydroxyacetate (20): A mixture of *tert*-butyl carbamate (13.54 g, 0.116 mol) and methyl glyoxylate methyl hemiacetal (15.27 g, 12.66 mL, 0.127 mol) in cyclohexane (150 mL) was refluxed in a Dean Stark apparatus for 15.5 h. After cooling to room temperature a yellow oil was formed on the bottom of the flask, which solidified upon standing overnight. The precipitate was collected by filtration and washed twice with cyclohexane yield **20** as a white solid (18.68 g, 91.0 mmol, 78.5%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.73 (br. s, 1 H), 5.44 (br. s, 1 H), 3.84 (s, 3 H), 3.78 (br. s 1 H), 1.46 (s, 9 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.12, 155.02, 81.02, 73.23, 53.02, 28.19 ppm. IR (neat): $\tilde{\nu}$ = 3373, 2981, 1714, 1516, 1442, 1369, 1252, 1159, 1056, 913, 856 cm^{-1}. HRMS(FAB): calcd. for $[C_8H_{15}NO_5 + H]^+$: 206.1028, found: 206.1027; R_f(PE/EtOAc, 1:1) = 0.31.

Methyl 2-Acetoxy-2-(*tert*-butoxycarbonylamino)acetate (21): A solution of **20** (25.55 g, 0.125 mol) and pyridine (0.30 mL, 3.71 mmol) in Ac_2O (150 mL) was stirred for 19 h. Volatiles were co-evaporated with toluene (3×) to give **21** as a yellow oil (30.89 g, 0.125 mmol, 99%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.22 (br. s, 1 H), 5.92 (br. s, 1 H), 3.81 (s, 3 H), 2.11 (s 3 H), 1.46 (s, 9 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.26, 167.09, 153.94, 81.25, 74.30, 53.11, 28.08, 20.63 ppm. IR (neat): $\tilde{\nu}$ = 3358, 2980, 1730, 1511, 1439, 1371, 1231, 1159, 1030, 859 cm^{-1}. HRMS(FAB): calcd. for $[C_{10}H_{17}NO_6 + H]^+$: 248.1134, found: 248.1135; R_f(PE/EtOAc, 1:1) = 0.60.

Methyl 2-(*tert*-Butoxycarbonylamino)-3-methylenepent-4-enoate (22): To a stirred solution of **21** (1.50 g, 6.07 mmol) and allenyltrimethylsilane[14,16] (1.53 g, 1.21 mmol) in CH_2Cl_2 (30 mL) at –78 °C was added slowly $BF_3{\cdot}OEt_2$ (1.53 mL, 1.21 mmol). The resulting yellow reaction mixture was warmed slowly to –20 °C, carefully monitored by TLC (PE/EtOAc, 2:1). After 4 h the solution was poured onto a saturated aqueous $NaHCO_3$ solution and extracted with EtOAc (3×). The organic layer was washed with brine, dried with Na_2SO_4, filtered, and concentrated in vacuo to give crude **22** in 1.26 g as a yellow oil. A small sample was purified by column chromatography (PE/EtOAc, 4:1) for analysis. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.32 (dd, J = 11.3, 17.9 Hz, 1 H), 5.46 (d, J = 17.7 Hz, 1 H), 5.28 (s, 1 H), 5.24 (m, 2 H), 5.19 (d, J = 11.3 Hz, 1 H), 5.08 (d, J = 7.7 Hz, 1 H), 3.75 (s, 3 H), 1.44 (s, 9 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.86, 157.64, 142.09, 135.27, 118.53, 115.78, 80.03, 53.22, 53.04, 28.19 ppm. IR (neat): $\tilde{\nu}$ = 3364, 2980, 1714, 1504, 1441, 1369, 1251, 1160, 1057, 916, 856 cm^{-1}. HRMS(FAB): calcd. for $[C_{12}H_{19}NO_4 + H]^+$: 242.1392, found: 242.1405; R_f(PE/EtOAc, 2:1) = 0.70.

(*S*)-Methyl 2-(*tert*-Butoxycarbonylamino)-3-methylenepent-4-enoate [(*S*)-22]: To a stirred solution of crude (*S*)-**23** (63 mg) in MeOH/

benzene (2 mL, 1:1) under an argon atmosphere was added drop wise TMS-diazomethane (2 M in hexanes, ca. 0.25 mL) until the yellow color persisted. After stirring at room temperature for 30 min, 5% AcOH/MeOH (1 drop) was added until the yellow color disappeared. Solid $NaHCO_3$ was added and the reaction mixture was concentrated in vacuo. The residue was purified by column chromatography (PE/EtOAc, 5:1, column rinsed with 2% Et_3N) to give (*S*)-**22** (26 mg, 0.11 mmol, 73% over 2 steps) as a colorless oil. ^1H NMR (400 MHz, $CDCl_3$): δ = 6.33 (dd, J = 11.1, 17.7 Hz, 1 H), 5.46 (d, J = 17.7 Hz, 1 H), 5.29 (s, 1 H), 5.26 (m, 1 H), 5.24 (s, 1 H), 5.20 (d, J = 11.1 Hz, 1 H), 5.09 (d, J = 8.0 Hz, 1 H), 3.76 (s, 3 H), 1.45 (s, 9 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 171.67, 154.96, 142.18, 135.28, 118.54, 115.81, 80.18, 54.73, 52.61, 28.29 ppm. IR (neat): $\tilde{\nu}$ = 2978, 1749, 1706, 1491, 1368, 1157 cm^{-1}. HRMS(FAB): calcd. for $[C_{12}H_{19}NO_4 + H]^+$: 242.1392, found: 242.1396.

2-(*tert*-Butoxycarbonylamino)-3-methylenepent-4-enoic Acid (23): To a stirred solution of crude **21** (2.0 g, 8.29 mmol) in THF (100 mL) at 0 °C was added drop wise a solution of LiOH (0.596 g, 24.9 mmol) in H_2O (100 mL). The slight yellow mixture was stirred at 0 °C for 4 h when it was poured on 5% $KHSO_4$ and extracted with EtOAc (3×). The organic layer was washed with brine, dried with Na_2SO_4, filtered, and concentrated in vacuo to yield **18** in 95% (1.78 g, 7.84 mmol) as a yellow oil. ^1H NMR (400 MHz, $CDCl_3$): δ = 9.42 (br. s, 1 H), 6.37 (dd, J = 11.2, 17.6 Hz, 1 H), 5.50 (d, J = 17.6 Hz, 1 H), 5.33 (s, 2 H), 5.21 (m, 1 H), 5.12 (d, J = 7.4 Hz, 1 H), 4.91 (d, J = 4.4 Hz, 1 H), 1.45 (s, 9 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 174.95, 154.95, 141.73, 135.05, 118.67, 115.80, 80.26, 54.55, 28.12 ppm. IR (neat): $\tilde{\nu}$ = 3311, 2979, 1723, 1504, 1394, 1369, 1250, 1162, 1056, 913 cm^{-1}. HRMS(FAB): calcd. for $[C_{11}H_{17}NO_4 + H]^+$: 228.1236, found: 228.1233; R_f(EtOAc/MeOH/AcOH, 9:1:0.01) = 0.57.

(*S*)-2-(*tert*-Butoxycarbonylamino)-3-methylenepent-4-enoic Acid [(*S*)-23]: To a stirred solution of (*S*)-**1a** (19 mg, 0.15 mmol) in THF/H_2O (2 mL, 1:1) were added subsequently Boc_2O (65 mg, 0.30 mmol) and $NaHCO_3$ (32 mg, 0.38 mmol). The reaction mixture was stirred for 2 h at room temperature after which all volatiles were removed in vacuo. The residue was acidified to pH 3–4 with 10% citric acid solution and extracted with EtOAc (4×). The combined organic layers were washed with brine, dried with $MgSO_4$, filtered, and concentrated in vacuo to yield (*S*)-**23** (63 mg) as a colorless oil which was used as such in the next step.

***rac*-2-Amino-3-methylenepent-4-enoic Acid TFA Salt (*rac*-1a):** To a stirred solution of **23** (0.225 g, 0.99 mmol) in $CHCl_3$ (3 mL) was added TFA (3 mL), After 45 min the brown mixture was concentrated in vacuo to yield a brown oil (0.216 g, 0.896 mmol, 90.4%). ^1H NMR (500 MHz, D_2O): δ = 6.31 (dd, J = 11.2, 17.8 Hz, 1 H), 5.46 (s, 1 H), 5.33 (d, J = 17.8 Hz, 1 H), 5.32 (s, 1 H), 5.18 (d, J = 11.2 Hz, 1 H), 4.64 (s, 1 H) ppm. ^{13}C NMR (100 MHz, D_2O): δ = 170.51, 162.67 (q), 138.03, 134.05, 122.22, 116.66, 53.02 ppm. CF_3COO^- not observed. IR (neat): $\tilde{\nu}$ = 1737, 1657, 1515, 1434, 1145, 1040, 921 cm^{-1}.

***tert*-Butyl *N*-(1-Amino-3-methylene-1-oxopent-4-en-2-yl)carbamate (24):** To a stirred solution of crude **23** (1.63 g, 7.18 mmol) at –40 °C in THF (15 mL) were added *N*-methylmorpholine (1.18 mL, 10.8 mmol) and isobutyl chloroformate (0.977 mL, 7.54 mmol). The resulting brown-grey suspension was stirred at –20 °C for 10 min when NH_4OH (25%, 3.35 mL, 21.5 mmol) was slowly added. The reaction temperature was kept between –15 and –25 °C for 4.5 h when the orange-brown solution was poured onto a saturated aqueous $NaHCO_3$ solution and extracted with EtOAc (3×). The organic layer was washed with brine, dried with Na_2SO_4, filtered, and concentrated in vacuo to give a yellowish solid which was purified by column chromatography (PE/EtOAc, 1:2) to give **24** as a white powder (1.23 g, 5.46 mmol, 76%). ^1H NMR (400 MHz, $CDCl_3$): δ = 6.37 (dd, J = 11.2, 17.8 Hz, 1 H), 5.91 (br. s, 1 H), 5.62 (br. s, 1 H), 5.57 (br. s, 1 H), 5.47 (d, J = 17.8 Hz, 1 H), 5.38 (m, 2 H), 5.24 (d, J = 11.2 Hz, 1 H), 4.97 (br. d, J = 4.9 Hz, 1 H), 1.46 (s, 9 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 172.39, 155.29, 142.98, 135.39, 119.06, 115.94, 80.13, 55.92, 28.30 ppm. IR (neat): $\tilde{\nu}$ = 3329, 2979, 1680, 1503, 1251, 1167, 914 cm^{-1}. HRMS(FAB): calcd. for $[C_{11}H_{18}N_2O_3 + H]^+$: 227.1396, found: 227.1388; R_f(EtOAc) = 0.43.

2-Amino-3-methylenepent-4-enamide, TFA Salt (25): To a stirred solution of **24** (2.68 g, 11.8 mmol) in $CHCl_3$ (25 mL) was added TFA (25 mL). The resulting yellow solution was stirred at room temperature for 30 min when it was co-evaporated with toluene (3×) to afford **25** as an off-white solid in 97% yield (2.75 g, 11.4 mmol). ^1H NMR (500 MHz, D_2O): δ = 6.33 (dd, J = 11.2, 17.8 Hz, 1 H), 5.55 (s, 1 H), 5.41 (s, 1 H), 5.36 (d, J = 17.8 Hz, 1 H), 5.23 (d, J = 11.2 Hz, 1 H), 4.77 (s, 1 H) ppm. ^{13}C NMR (100 MHz, D_2O): δ = 170.13, 163.09 (q), 138.08, 133.55, 122.79, 116.67, 53.37 ppm. CF_3COO^- not observed. IR (neat): $\tilde{\nu}$ = 1660, 1601, 1516, 1395, 1143, 930, 840 cm^{-1}. HRMS(FAB): calcd. for $[C_6H_{10}N_2O + H]^+$: 127.0871, found: 127.0871.

(*S*)-2-Amino-3-methylenepent-4-enoic Acid [(*S*)-1a]: To a suspension of racemic amide-TFA salt **25** (0.731 g, 3.04 mmol) in water (3 mL) was added drop wise an aqueous KOH solution until pH 9. A 80 mmol/L solution of $MnSO_4$ (88 μL, 0.007 mmol) was added and the total volume of the solution was brought up to 7 mL. The solution was brought to 40 °C before adding the enzyme (a cell free extract of a recombinant *Escherichia coli* strain overexpressing the *P. putida* L-aminopeptidase gene,[19] 0.25 mL) and the mixture was shaken at 40 °C for 16 h. After cooling to room temperature the pH was brought to pH 4 with H_2SO_4 and the mixture was filtered through Celite. The mixture was brought back to pH 9 with a KOH solution when benzaldehyde (165 μL, 1.63 mmol) was added. The mixture was stirred for 2 h at room temperature when it was extracted with dichloromethane (3 × 20 mL) to extract the Schiff base of the D-amide. The combined organic phases were dried with Na_2SO_4 and concentrated in vacuo. The L-acid in the aqueous phase was purified via Ion Exchange Chromatography, using the strong basic resin Amberlite IRA-410 to give the desired amino acid (*S*)-**1a** (146 mg, 1.15 mmol 37.7%) as a white solid. ^1H NMR (400 MHz, D_2O): δ = 6.31 (dd, J = 11.2, 17.8 Hz, 1 H), 5.41 (s, 1 H), 5.32 (m, 2 H), 5.17 (d, J = 11.2 Hz, 1 H), 4.41 (s, 1 H) ppm. ^{13}C NMR (125 MHz, D_2O): δ = 139.90, 134.57, 121.85, 116.11, 55.35 ppm. COO^- not observed. HRMS(FAB): calcd. for $[C_6H_9NO_2 + H]^+$: 128.0712, found: 128.0712. $[\alpha]_D^{20}$ = +102 (c = 0.05, D_2O); m.p. > 250 °C (dec.).

(*S*)-Methyl 5-Amino-2-(*tert*-butoxycarbonylamino)-5-oxopentanoate (Boc-Glutamine Methyl Ester, 9): TMS-CHN_2 (2.0 M in hexanes, 40.6 mL, 81.2 mmol) was added slowly via a pressure equalising dropping funnel to a stirred solution of Boc-glutamine (8.00 g, 32.5 mmol) in MeOH/toluene (30 mL/75 mL). After 0.5 h acetic acid was added to the yellow solution to quench excess TMS-CHN_2 and the colorless mixture was concentrated in vacuo. The crude product was precipitated from TBME/pentane to afford **9** as a white solid (7.41 g, 28.5 mmol, 87.5%). ^1H NMR (500 MHz, $CDCl_3$): δ = 6.10 (br. s, 1 H), 5.39 (br. s, 1 H), 5.31 (br. d, J = 7.5 Hz, 1 H), 4.35 (br. s, 1 H), 3.77 (s, 3 H), 2.34 (m, 2 H), 2.21 (m 1 H), 1.94 (m, 1 H), 1.46 (s, 9 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 174.56, 172.75, 155.87, 80.18, 52.95, 52.46, 31.83, 28.82, 28.28 ppm. IR (neat): $\tilde{\nu}$ = 3350, 2979, 1668, 1520, 1454,

1367, 1167, 1054, 918 cm^{-1}. HRMS(FAB): calcd. for $[C_{11}H_{20}N_2O_5+H]^+$: 261.1450, found: 261.1448.

(2*S*)-Methyl 2-(*tert*-Butoxycarbonylamino)-5-(1-hydroxy-2-methoxy-2-oxoethylamino)-5-oxopentanoate (26): A stirred solution of **9** (2.50 g, 9.61 mmol) and methyl glyoxylate methyl hemiacetal (1.91 mL, 19.2 mmol) was refluxed for 19 h in $CHCl_3$ (90 mL). The reflux condenser was placed on a pressure equalising dropping funnel filled with 4 Å mol. sieves. After cooling to room temperature the yellow solution was washed with a saturated aqueous $NaHCO_3$ solution and brine. The organic layer was dried with Na_2SO_4, filtered, and concentrated in vacuo. The crude product was purified by column chromatography (EtOAc) to afford **26** as thick yellow oil in 58% yield (1.94 g, 5.58 mmol) in a 1:1 mixture of diastereomers. ^1H NMR (400 MHz, $CDCl_3$): δ = 7.53 (br. d, J = 6.6 Hz, 0.5 H), 7.45 (br. s, J = 6.6 Hz, 0.5 H), 5.63 (d, J = 7.4 Hz, 0.5 H), 5.57 (d, J = 7.4 Hz, 0.5 H), 5.40 (br. s, 1 H), 4.61 (br. s, 1 H), 4.34 (m, 1 H), 3.85 (s, 3 H), 3.76 (s, 3 H), 2.36 (m, 2 H), 2.23 (m, 1 H), 1.92 (m, 1 H), 1.45 (s, 9 H) ppm.[27] ^{13}C NMR (100 MHz, $CDCl_3$): δ = 172.93, 172.63, 169.94, 169.87, 155.91, 80.52, 72.35, 72.22, 53.25, 53.20, 52.59, 32.16, 29.05, 28.81, 28.28 ppm. IR (neat): $\tilde{\nu}$ = 3332, 2980, 1746, 1694, 1531, 1443, 1166 cm^{-1}. HRMS(FAB): calcd. for $[C_{14}H_{24}N_2O_8 + H]^+$: 349.1611, found: 349.1592; R_f(EtOAc) = 0.38.

(2*S*)-Methyl 5-(1-Acetoxy-2-methoxy-2-oxoethylamino)-2-(*tert*-butoxycarbonylamino)-5-oxopentanoate (27): A mixture of **26** (1.14 g, 3.27 mmol) and pyridine (0.1 mL, 1.24 mmol) in Ac_2O (10 mL) was stirred for 20 h when the orange solution was concentrated in vacuo and co-evaporated with toluene (3×) to give **27** as a sticky yellow oil in 97% (1.24 g, 3.18 mmol). ^1H NMR (500 MHz, $CDCl_3$): δ = 6.41 (m, 1 H), 5.25 (br. s, 1 H), 4.35 (br. s, 1 H), 3.83 (s, 3 H), 3.77 (s, 3 H), 2.36 (m, 2 H), 2.23 (m, 1 H), 2.14 (s, 3 H), 1.96 (m, 1 H), 1.46 (s, 9 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 172.64, 171.94, 170.34, 170.28, 167.19, 155.69, 80.19, 72.13, 53.25, 52.72, 52.48, 31.97, 28.26, 20.62 ppm. IR (neat): $\tilde{\nu}$ = 3344, 2979, 1747, 1519, 1439, 1369, 1218, 1166, 1032, 917 cm^{-1}. HRMS(FAB): calcd. for $[C_{16}H_{26}N_2O_9 + H]^+$: 391.1717, found: 391.1726; R_f(PE/EtOAc, 1:2) = 0.38.

Methyl 2-[(*S*)-4-(*tert*-Butoxycarbonylamino)-5-methoxy-5-oxopentanamido]-3-methylenepent-4-enoate [(*S*)-28]: To a stirred solution of **27** (0.475 g, 1.22 mmol) and allenylmethyltrimethylsilane (0.307g, 2.43 mmol) in CH_2Cl_2 (6 mL) at –78 °C, was added drop wise $BF_3{\cdot}OEt_2$ (0.461 g, 3.65 mmol). The resulting mixture was warmed slowly following the reaction with TLC. After 5.5 h (T = –25 °C), the yellow solution was poured onto a saturated aqueous $NaHCO_3$ solution and extracted with EtOAc (3×). The organic layer was washed with brine, dried with Na_2SO_4, filtered, and concentrated in vacuo to afford a yellow oil. Purification by column chromatography (PE/EtOAc, 1:1) afforded (*S*)-**28** in 26% (0.123 g, 0.32 mmol) as a colorless oil. ^1H NMR (500 MHz, $CDCl_3$): δ = 6.92 (d, J = 6.2 Hz, 0.5 H), 6.62 (d, J = 5.3 Hz, 0.5 H), 6.32 (ddd, J = 2.7, 11.1, 17.7 Hz, 1 H), 5.44 (d, J = 17.7 Hz, 1 H), 5.37 (dd, J = 4.5, 7.8 Hz, 1 H), 5.33 (br. s, 1 H), 5.30 (d, J = 2.0 Hz, 1 H), 5.25 (d, J = 8.6 Hz, 1 H), 5.18 (d, J = 11.1 Hz, 1 H), 4.42 (dt, J = 4.4, 9.2 Hz, 0.5 H), 4.28 (dt, J = 4.5, 8.6 Hz, 0.5 H), 3.74 (s, 3 H), 3.73 (s, 1.5 H), 3.72 (s, 1.5 H), 2.33 (m, 2 H), 2.18 (m, 1 H), 1.91 (m, 1 H), 1.43 + 1.42 (s, 9 H, rotamers) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 173.06, 172.97, 171.92, 171.61, 171.53, 156.10, 155.91, 142.00, 135.53, 119.27, 119.18, 116.16, 116.14, 80.35, 80.31, 53.61, 53.08, 53.00, 52.93, 52.89, 52.68, 32.56, 32.42, 29.49, 28.95, 28.50 ppm. IR (neat): $\tilde{\nu}$ = 3327, 2979, 2955, 1746, 1713, 1519, 1367, 1208, 1168, 914 cm^{-1}. HRMS(FAB): calcd. for $[C_{18}H_{28}N_2O_7 + H]^+$: 385.1975, found: 385.1981; R_f(PE/EtOAc, 1:1) = 0.28.

Methyl 2-[(*S*)-4-(*tert*-Butoxycarbonylamino)-5-methoxy-5-oxopentanamido]pent-4-enoate (29): To a stirred solution of Boc-Glu-OMe (1.00 g, 3.83 mmol) at –20 °C in THF (10 mL) were added NMM (0.644 mL, 5.74 mmol) and IBC (0.521 mL, 4.02 mmol). The mixture was stirred at –20 °C for 10 min when an aqueous solution (1 mL) of allylglycine methyl ester HCl salt (0.697 g, 4.21 mmol) and DIPEA (0.800 mL, 4.59 mmol) was slowly added. The resulting mixture was stirred at –20 °C for 2 h before it was warmed to room temperature in 19h. The mixture was poured on 5% $KHSO_4$ and extracted with EtOAc (3×). The organic layer was washed with brine, dried with Na_2SO_4, filtered, and concentrated in vacuo to afford a yellow oil. Purification by column chromatography (PE/EtOAc, 1:1) afforded **29** in 46.5% (0.662 g, 1.78 mmol 1:1 mixture of diastereomers). ^1H NMR (500 MHz, $CDCl_3$): δ = 6.77 (br. s, 0.5 H), 6.46 (br. s, 0.5 H), 5.69 (m, 1 H), 5.33 (m, 1 H), 5.13 (m, 2 H), 4.65 (m, 1 H), 4.39 (br. s, 0.5 H), 4.29 (br. s, 0.5 H), 3.73 (s, 6 H), 2.57 (m, 1 H), 2.49 (m, 1 H), 2.30 (m, 2 H), 2.17 (m, 1 H), 1.91 (m, 1 H), 1.42 (s, 9 H) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 173.01, 172.96, 172.45, 172.39, 172.07, 171.77, 156.09, 155.91, 132.59, 132.54, 119.33, 119.26, 80.31, 80.24, 53.12, 53.06, 52.63, 52.60, 52.53, 52.11, 51.98, 36.62, 36.47, 32.57, 32.47, 28.43 ppm. IR (neat): $\tilde{\nu}$ = 3326, 2979, 1745, 1714, 1659, 1525, 1167 cm^{-1}. HRMS(FAB): calcd. for $[C_{17}H_{28}N_2O_7 + H]^+$: 373.1975, found: 373.1982; R_f(PE/EtOAc, 1:2) = 0.40.

2-[(*S*)-4-(*tert*-Butoxycarbonylamino)-4-carboxybutanamido]pent-4-enoic Acid (30): To a stirred solution of **29** (50 mg, 0.134 mmol) in THF (1.5 mL) at 0 °C was added drop wise a pre-cooled aqueous (1.5 mL) solution of LiOH (12.9 mg, 0.537 mmol). The resulting mixture was stirred at 0 °C for 4.25 h when it was poured on 5% $KHSO_4$ and extracted with EtOAc (3×). The organic layer was washed with brine, dried with Na_2SO_4, filtered, and concentrated in vacuo to afford **30** as a colorless oil in 84% (39 mg, 0.113 mmol, 1:1 mixture of diastereomers). ^1H NMR (400 MHz, MeOD): δ = 5.81 (tdd, J = 7.0, 10.1, 17.2 Hz, 1 H), 5.16 (dd, J = 1.7, 17.1 Hz, 1 H), 5.11 (dd, J = 1.0, 10.2 Hz, 1 H), 4.48 (ddd, J = 2.8, 5.1, 7.9 Hz, 1 H), 4.13 (dt, J = 4.9, 8.6 Hz, 1 H), 2.62 (m, 1 H), 2.47 (m, 1 H), 2.38 (t, J = 7.5 Hz, 2 H), 2.16 (m, 1 H), 1.92 (m, 1 H), 1.46 (s, 9 H) ppm. ^{13}C NMR (100 MHz, MeOD): δ = 175.66, 175.61, 174.98, 174.90, 174.76, 174.74, 158.11, 134.54, 118.78, 118.77, 80.65, 54.53, 54.46, 53.44, 37.01, 36.99, 33.20, 33.09, 30.98, 28.93, 28.21 ppm. IR (neat): $\tilde{\nu}$ = 3328, 2981, 2506, 1727, 1537, 1394, 1369, 1246, 1163, 1056 cm^{-1}. HRMS(FAB): calcd. for $[C_{15}H_{24}N_2O_7 + H]^+$: 345.1662, found: 345.1660; R_f(EtOAc/AcOH, 1:0.02) = 0.05.

2-[(*S*)-4-Amino-4-carboxybutanamido]pent-4-enoic Acid, TFA Salt (31): To a stirred solution of **30** (39 mg, 0.113 mmol) in $CDCl_3$ (1 mL) was added TFA (1 mL). The resulting slight brown solution was stirred for 1h, when all volatiles were removed by co-evaporation with toluene to give **31** as brown oil in 84% (34 mg, 0.949 mmol, 1:1 mixture of diastereomers). ^1H NMR (400 MHz, D_2O): δ = 5.71 (tdd, J = 7.1, 10.1, 17.2 Hz, 1 H), 5.09 (m, 1 H), 4.36 (td, J = 5.2, 7.6 Hz, 1 H), 3.97 (t, J = 6.5 Hz, 1 H), 2.54 (m, 1 H), 2.44 (m, 3 H), 2.14 (m, 2 H) ppm. ^{13}C NMR (125 MHz, D_2O): δ = 174.96, 174.06, 173.99, 171.47, 171.46, 162.72 (q), 132.48, 118.62, 52.40, 52.36, 52.20, 52.14, 34.68, 34.62, 30.64, 30.50, 25.42, 25.38 ppm. CF_3COO^- not observed. IR (neat): $\tilde{\nu}$ = 1728, 1642, 1536, 1148, 930 cm^{-1}.

Methyl 2-Amino-3-methylenepent-4-enoate, TFA Salt (*rac*-32): To a solution of **22** (1.35 g, 5.57 mmol) in $CHCl_3$ (5 mL) was added TFA (10 mL). The mixture was stirred for 1.5 h when it was concentrated in vacuo and co-evaporated with toluene (3×) to yield *rac*-**32** as a brown solid in 99% (1.42 g, 5.57 mmol). ^1H NMR (400 MHz,

D_2O): δ = 6.37 (dd, J = 11.2, 17.8 Hz, 1 H), 5.57 (s, 1 H), 5.38 (d, J = 17.8 Hz, 1 H), 5.27 (d, J = 11.2 Hz, 1 H), 4.93 (s, 1 H), 3.77 (s, 3 H) ppm. ^{13}C NMR (100 MHz, D_2O): δ = 169.32, 162.79 (q), 137.70, 133.99, 122.17, 116.82, 53.94, 52.91 ppm. CF_3COO^- not observed. IR (neat): $\tilde{\nu}$ = 1737, 1655, 1540, 1433, 1178, 1129, 904 838 cm^{-1}. HRMS(FAB): calcd. for $[C_7H_{11}NO_2 + H]^+$: 142.0868, found: 142.0870.

(*S*)-Methyl 2-Amino-3-methylenepent-4-enoate, TFA Salt [(*S*)-32]: To a stirred solution of (*S*)-**22** (28 mg, 0.12 mmol) in $CHCl_3$ (0.4 mL) at 0 °C was added TFA (0.4 mL). The solution was stirred at room temperature for 10 min, when it was concentrated in vacuo and co-evaporated with toluene (3×) to give (*S*)-**32** as a colorless oil which was used as such in the next step.

(*S*)-Methyl 2-[4-(*tert*-Butoxycarbonylamino)-5-methoxy-5-oxopentanamido]-3-methylenepent-4-enoate [(*S*,*S*/*R*)-28]: To a stirred solution of Boc-Glu-OMe (0.563 g, 2.16 mmol) at –20 °C in THF (5.6 mL) were added NMM (0.275 mL, 2.45 mmol) and IBC (0.294 mL, 2.26 mmol). The mixture was stirred at –20 °C for 10 min when an mixture of *rac*-**32** (0.500 g, 1.96 mmol) and $NaHCO_3$ (0.206 mL, 2.45 mmol) in H_2O/THF (2 mL, 1:1) was slowly added. The resulting mixture was warmed to room temperature in 18 h, when all volatiles were evaporated. The residue was taken up in EtOAc and washed with saturated $NaHCO_3$, brine, dried with Na_2SO_4, filtered, and concentrated in vacuo to afford a brown foam. Purification by column chromatography (PE/EtOAc, 1:1) afforded (*S*,*S*/*R*)-**28** in 56% (0.421 g, 1.10 mmol 1:1 mixture of diastereomers). Analytical data were identical to those reported earlier in this paper.

(*S*)-Methyl 2-[(*S*)-4-(*tert*-Butoxycarbonylamino)-5-methoxy-5-oxopentanamido]-3-methylenepent-4-enoate [(*S*,*S*)-28]: To a stirred solution of Boc-Glu-OMe (63 mg, 0.24 mmol) in THF (1 mL) at –20 °C under an argon atmosphere were added NMM (32 μL, 0.29 mmol) and IBC (34 μL, 0.26 mmol). The suspension was stirred at –20 °C for 20 min when a precooled solution (0 °C) of crude (*S*)-**32** (0.12 mmol) in THF (0.5 mL)/H_2O (0.5 mL)/$NaHCO_3$ (1 M, 0.15 mL) was added drop wise. The resulting mixture was warmed to room temperature in 3 h, and stirred at room temperature for 20 h. THF was evaporated in vacuo and the residue was diluted with a saturated aqueous $NaHCO_3$ solution and extracted with EtOAc (4×). The combined organic layers were washed with brine, dried with $MgSO_4$, filtered, and concentrated in vacuo. The crude product (58 mg) was purified by column chromatography (PE/EtOAc, 1:1, column rinsed with 2% Et_3N) to give (*S*,*S*)-**28** (31 mg, 0.081 mmol, 67% over 2 steps) as a white needles (Et_2O/pentane). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.61 (br. d, J = 6.8 Hz, 1 H), 6.34 (dd, J = 11.1, 17.7 Hz, 1 H), 5.46 (d, J = 17.7 Hz, 1 H), 5.38 (d, J = 7.7 Hz, 1 H), 5.31 (s, 1 H), 5.29 (br. s, 1 H), 5.27 (s, 1 H), 5.20 (d, J = 11.1 Hz, 1 H), 4.30 (dt, J = 4.5, 8.3 Hz, 1 H), 3.76 (s, 3 H), 3.74 (s, 3 H), 2.35 (t, J = 7.4 Hz, 2 H), 2.20 (m, 1 H), 1.93 (td, J = 6.8, J = 14.6 Hz, 1 H), 1.45 (s, 9 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 172.73, 171.37, 171.30, 155.68, 141.77, 135.29, 118.97, 115.94, 80.12, 53.39, 52.86, 52.69, 52.44, 32.21, 28.76, 28.28 ppm. HRMS(FAB): calcd. for $[C_{18}H_{28}N_2O_7 + H]^+$: 385.1975, found: 385.1981. $[\alpha]_D^{20}$ = +72.6 (c = 0.5, $CHCl_3$); R_f(PE/EtOAc, 1:2) = 0.45; m.p. 108–112 °C.

(*S*)-2-[4-(*tert*-Butoxycarbonylamino)-4-carboxybutanamido]-3-methylenepent-4-enoic Acid [(*S*,*S*/*R*)-33]: To a stirred solution of (*S*,*S*/*R*)-**28** (0.390 g, 1.02 mmol) in THF (10 mL) at 0 °C was added drop wise a precooled solution of LiOH (97 mg, 4.06 mmol) in H_2O (10 mL). The resulting yellowish mixture was stirred at 0 °C for 3 h when it was poured on 5% $KHSO_4$ and extracted with EtOAc (3×). The organic layer was washed with brine, dried with Na_2SO_4, filtered, and concentrated in vacuo to afford (*S*,*S*/*R*)-**33** as a fluffy white powder in 80% yield (0.288 g, 0.808 mmol, 1:1 mixture of diastereomers). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.43 (dd, J = 11.1, 17.6 Hz, 1 H), 5.83 (br. s, 1 H), 5.43 (d, J = 17.6 Hz, 1 H), 5.36 (s, 1 H), 5.29 (m, 2 H), 5.18 (d, J = 11.1 Hz, 1 H), 2.38 (m, 2 H), 2.17 (m, 1 H), 1.90 (m, 1 H), 1.46 (s, 9 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 174.30, 173.32, 173.27, 172.43, 172.40, 156.71, 142.24, 135.88, 135.74, 117.83, 114.24, 114.21, 79.16, 51.52, 48.45, 31.61, 31.52, 27.32 ppm. IR (neat): $\tilde{\nu}$ = 3313, 2980, 1714, 1694, 1519, 1249, 1165, 1055, 913 cm^{-1}. HRMS(FAB): calcd. for $[C_{16}H_{24}N_2O_7 + H]^+$: 357.1662, found: 357.1663; R_f(EtOAc/AcOH, 1:0.02) = 0.10.

(*S*)-2-[(*S*)-4-(*tert*-Butoxycarbonylamino)-4-carboxybutanamido]-3-methylenepent-4-enoic Acid [(*S*,*S*)-33]: To a stirred solution of (*S*,*S*)-**28** (25 mg, 0.065 mmol) in THF (0.6 mL) at 0 °C was added a precooled (0 °C) solution of LiOH (6.2 mg, 0.260 mmol) in H_2O. The resulting yellow solution was stirred at 0 °C for 1.5 h when it was poured on 5% $KHSO_4$ and extracted with EtOAc (3×). The organic layer was washed with brine, dried with Na_2SO_4, filtered, and concentrated in vacuo to afford (*S*,*S*)-**33** as a colorless oil in 99% yield (23 mg, 0.0645 mmol). ^{1}H NMR (500 MHz, MeOD): δ = 6.41 (dd, J = 11.1, 17.6 Hz, 1 H), 5.41 (d, J = 17.6 Hz, 1 H), 5.34 (s, 1 H), 5.28 (m, 2 H), 5.17 (d, J = 11.1 Hz, 1 H), 4.09 (dd, J = 4.5, 9.1 Hz, 1 H), 2.37 (t, J = 7.3 Hz, 2 H), 2.14 (m, 1 H), 1.89 (m, 1 H), 1.44 (s, 9 H) ppm. ^{13}C NMR (125 MHz, MeOD): δ = 174.46, 173.50, 172.55, 156.94, 142.41, 142.39, 136.07, 118.05, 118.00, 114.49, 79.39, 54.64, 53.26, 53.22, 31.73, 29.72, 27.54 ppm. IR (neat): $\tilde{\nu}$ = 3327, 2977, 2930, 1720, 1657, 1525, 1394, 1249, 1164, 1050, 914 cm^{-1}. HRMS(FAB): calcd. for $[C_{16}H_{24}N_2O_7 + H]^+$: 357.1662, found: 357.1660. $[\alpha]_D^{20}$ = +45.9 (c = 1.15, MeOH), R_f(EtOAc/AcOH, 1:0.02) = 0.08.

(*S*)-2-(4-Amino-4-carboxybutanamido)-3-methylenepent-4-enoic Acid, TFA Salt [(*S*,*S*/*R*)-1b)]: To stirred solution of **33** (0.100 g, 0.281 mmol) in $CHCl_3$ (2 mL) was added TFA (2 mL). The resulting solution was stirred at room temperature for 45 min when it was co-evaporated with toluene to yield (*S*,*S*/*R*)-**1b** as a yellow oil (97 mg, 0.262 mmol, 93%, 1:1 mixture of diastereomers). ^{1}H NMR (500 MHz, D_2O): δ = 6.28 (dd, J = 11.1, 17.7 Hz, 1 H), 5.29 (s, 1 H), 5.20 (d, J = 17.7 Hz, 1 H), 5.16 (s, 1 H), 5.09 (d, J = 6.7 Hz, 1 H), 5.07 (s, 1 H) 3.91 (t, J = 6.5 Hz, 1 H), 2.40 (m, 2 H), 2.07 (m, 2 H) ppm. ^{13}C NMR (100 MHz, D_2O): δ = 174.00, 173.93, 173.86, 173.84, 171.35, 171.33, 162.79 (q), 140.46, 140.40, 135.22, 120.17, 120.09, 115.57, 53.88, 53.82, 52.10, 52.04, 30.69, 30.53, 25.40, 25.36 ppm. CF_3COO^- not observed. HRMS(FAB): calcd. for $[C_{13}H_{17}F_3N_2O_7 + H]^+$: 371.1066, found: 371.1097.

(*S*)-2-[(*S*)-4-Amino-4-carboxybutanamido]-3-methylenepent-4-enoic Acid, TFA Salt [(*S*,*S*)-1b]: To stirred solution of (*S*,*S*)-**33** (11 mg, 0.031 mmol) in $CHCl_3$ (0.2 mL) was added TFA (0.2 mL). The resulting solution was stirred at room temperature for 10 min when it was co-evaporated with toluene to yield (*S*,*S*)-**1b** as a yellow oil (11 mg, 0.031 mmol, 99%). ^{1}H NMR (400 MHz, D_2O): δ = 6.33 (dd, J = 11.1, 17.7 Hz, 1 H), 5.33 (s, 1 H), 5.24 (d, J = 17.8 Hz, 1 H), 5.20 (s, 1 H), 5.12 (m, 2 H), 3.90 (t, J = 6.6 Hz, 1 H), 2.43 (t, J = 7.3 Hz, 2 H), 2.09 (m, 2 H) ppm. ^{13}C NMR (125 MHz, D_2O): δ = 174.311, 174.26, 172.16, 163.18 (q), 140.71, 140.69, 135.56, 120.33, 115.72, 54.21, 52.76, 30.99, 25.79 ppm. CF_3COO^- not observed. IR (neat): $\tilde{\nu}$ = 2924, 2854, 1724, 1639, 1517, 1161, 915 cm^{-1}. HRMS(FAB): calcd. for $[C_{13}H_{17}F_3N_2O_7 + H]^+$ (TFA salt): 371.1066, found: 371.1097, HRMS(FAB): calcd. for $[C_{11}H_{16}N_2O_5 + H]^+$ (free amine): 257.1137, found: 257.1128, $[\alpha]_D^{20}$ = +35.5 (c = 0.55, D_2O).

Acknowledgments

The authors kindly acknowledge M. Boesten and I. Maes (DSM, Geleen, The Netherlands) for their efforts on the HPLC analysis of the amino acid resolutions. B. Dassen is thanked for his help on the Alcalase®-mediated resolution. This research was financially supported by the Council for Chemical Sciences of the Netherlands Organization for Scientific Research (NWO-CW).

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Received: October 26, 2007
Published Online: December 11, 2007

