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A Stereoselective Entry to Enantiopure (*S*)-2-Amino-2-methyl-5-arylpent-4-ynoic Acids and Evaluation of Their Inhibitory Activity against Bacterial Collagenase G

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Abstract: Nowadays, amino acids (AAs) and peptides with bulky side chains hold significant interest for organic synthesis and the modern pharma industry. Non-proteinogenic (or unnatural) AAs are key building blocks used for obtaining pharmaceutically relevant peptides and for the development of chiral molecular catalysts, and they are extensively used in the total synthesis of complex natural compounds. Thus, an elaboration of cost-effective methods for the preparation of novel unnatural AAs to increase their structural diversity is highly desirable. In this context, herein, we present an asymmetric metal-templated synthesis of a number of enantiomerically pure (*S*)-2-amino-2-methyl-5-arylpent-4-ynoic acids starting from commercially available reagents and Belokon's chiral auxiliary (*S*)-BPB, namely *(S)*-2-(*N*-benzylprolyl)aminobenzophenone. The construction of a chiral Ni(II) complex from alanine (Ala) and the subsequent propargylation, arylation by the Sonogashira cross-coupling reaction using various aryl halides, and, finally, an acidic decomposition of the obtained complexes deliver to the target complex α,α-disubstituted AAs featuring a triple bond in a side chain. Next, the Fmoc-protected α-AAs and dipeptide were synthesized. Finally, we examined the obtained α-AAs and peptide as collagenase inhibitors.

Keywords: amino acids; asymmetric synthesis; Sonogashira cross-coupling; peptides; Ni(II) complex; triple bond

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1. Introduction

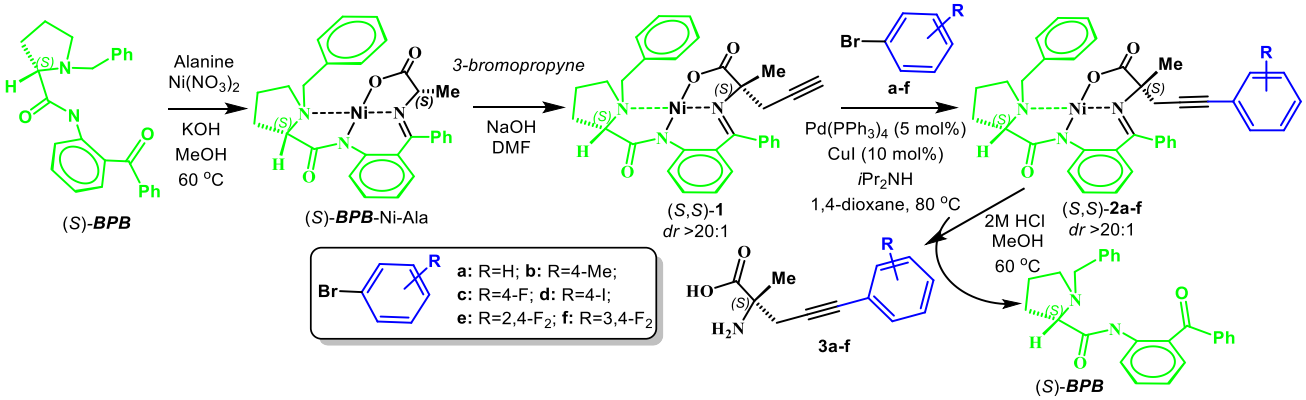
In contrast to the classical synthetic routes, modern chemistry focuses on the production of enantiomerically pure compounds and building blocks. Among them, to date, chiral amino acids (AAs) have a crucial role in medicinal chemistry and in drug design [1–7]. In addition, AAs have been widely applied in the development of chiral molecular catalysts [8–10] and in the total synthesis of complex natural products [11]. Therefore, either a chiral substrate, catalyst or even a chiral auxiliary are required to provide a high asymmetric induction in AA syntheses [12,13]. In this regard, chiral Ni(II) complexes based on chiral ligand—(*S*)- or (*R*)-*N*-(benzylprolyl)aminobenzophenone (BPB) and amino acids introduced by Belokon [14] were developed in the last decades as robust and easily available chiral starting materials for the synthesis of tailor-made AAs [15–22]. While the chiral auxiliary BPB reliably provides an asymmetric induction, a Ni ion organizes a proper geometry simultaneously serving as a protecting group for the *N*- and *O*-terminus of an α-AA and increasing the acidity of α-protons in the AA residue [15–22].

The use of this metal-templated strategy has some advantages over microbiological and enzymatic asymmetric synthetic protocols. In particular, the reactions can be carried out in concentrated solutions of commercially available organic solvents. The reactions are highly reproducible and the target α-AAs are easily separable from the reaction mixture. Overall, it is often difficult to find enzymatic protocols for the preparation of new unnatural AAs because of their unusual complex structure [23].

On the other hand, enantiopure α,α-disubstituted non-proteinogenic α-AAs have been broadly applied in the design of novel peptides and proteins with enhanced biological properties due to their stability towards racemization and restricted conformational flexibility [24]. Peptides are in high demand in such important areas as the agriculture, food and pharmaceutical industries [25–27]. Peptide therapeutics have attracted increasing interest in recent years due to the shift from small molecules (i.e., <500 Da) to pharmaceuticals with high molecular weight in the cases of new drug discovery that allow for overcoming resistance problems [28,29]. In particular, peptides can selectively interact with receptors, such as G protein-coupled receptors (GPCR), which is difficult in the case of small-molecule drugs [30].

Commonly, there are two synthetic protocols for constructing peptides used in academia and industry: liquid-phase peptide synthesis (LPPS) and solid-phase peptide synthesis (SPPS). Syntheses of peptides performed in solution require a careful choice of protecting groups and multiple challenging workups and isolation procedures [31,32]. The use of this methodology can be problematic for the synthesis of long and more complex peptides but remains valid for short peptides [33–38]. Merrifield introduced SPPS, greatly simplifying the removal of non-tethered entities like reagents and solvents. This approach opened the doors for automation as subsequent couplings could be iteratively performed by a defined set of unit operations. As a result, many challenging peptides have been synthesized using SPPS [39,40]. However, SPPS is still far from superior, especially in terms of greenness. A typical synthesis requires an excess of amino acids, coupling reagents and base to achieve high conversion at each step. Moreover, the synthesis of hydrophobic peptides poses additional challenges since aggregation of growing peptide chains is a common issue [41–44]. Given the importance of peptides, it is highly desirable to elaborate routes for the synthesis of new peptides.

Herein, we present a new and convenient synthesis of optically pure (*S*)-2-amino-2-methyl-5-arylpent-4-ynoic acids starting from a chiral alanine Ni(II) complex (*S*)-BPB-Ni-Ala through the propargylation and subsequent arylation by the Sonogashira cross-coupling reaction followed by an acidic decomposition of the synthesized complexes (Scheme 1). Next, the Fmoc-protected AAs and dipeptide were obtained for biological activity evaluation.



Scheme 1. Synthesis of the starting complex (*S*,*S*)-**1** and complexes (*S*,*S*)-**2a**–**f**, and the isolation of the target AAs (*S*)-**3**.

2. Materials and Methods

2.1. Instrumentation

All reactions were set up using standard Schlenk techniques and carried out under argon or nitrogen atmospheres using anhydrous solvents, unless otherwise noted. Anhydrous solvents, including DMA, 1,4-dioxane (99%+, Extra dry AcroSeal®), were purchased from Acros Organics and used as received. Commercially available chemicals were obtained from J&K, Across, TCI or Adamas and used as received unless otherwise stated. ^{1}H and ^{13}C NMR spectra were recorded using a Varian Mercury 300 spectrometer (Agilent, Santa Clara, CA, USA) (300 MHz for ^{1}H and 75.5 MHz for ^{13}C). Chemical shifts (δ) for ^{1}H and ^{13}C NMR spectra are given in ppm relative to TMS. The residual solvent signals were used as references for ^{1}H and ^{13}C NMR spectra and the chemical shifts converted to the TMS scale ($CDCl_3$): δ(H) = 7.26 ppm, δ(C) = 77.16 ppm; $(CD_3)_2SO$: δ(H) = 2.50 ppm, δ(C) = 39.52 ppm. The following abbreviations were used to explain the multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, b = broad. High-resolution mass spectra were recorded using a Bruker MicroTOF II instrument using the ESI ionization method (BRUKER DALTONIK GmbH, Bremen, Germany). The high-performance liquid chromatographic system used to determine the enantiomeric purity of the AAs was a Waters Alliance 2695e Separation Module HPLC system equipped with a PDA detector (Waters Corporation, Milford, MA, USA). The separation was accomplished in isocratic mode using a Nautilus-E 5μ 4.0 × 250 mm column (BioChimMac ST Company, Moscow, Russia) at 30 °C. The mobile phase consisted of methanol and monosodium phosphate buffer (25 mmol/L). The compound's enantiomeric excess was confirmed using chiral HPLC analysis of the isolated amino acids.

Reactions were monitored using thin-layer chromatography (TLC) carried out on 0.2 ± 0.03 mm using UV light (254 nm) as a visualizing agent and phosphomolybdic acid in ethanol or iodine.

2.2. Procedure for Synthesis of Complex **1**

Complex (*S*)-BPB-Ni-Ala (10 g, 1.1 mmol, 1.0 equiv.) was added to a stirred solution of propargyl bromide (1.8 mL, 2.3 mmol, 1.2 equiv.) and NaOH (2.35 g, 3.3 mmol, 3.0 equiv.) in DMF (30 mL). The mixture was stirred for 4 h at room temperature. After TLC analysis, the mixture was diluted with DCM and washed two times with water. The organic layer was dried over Na_2SO_4 and filtered. Afterwards, the solvent was removed under reduced pressure and the residue was purified using column chromatography (eluent: EtOAc) to obtain complex **1** as a red solid (7.28 g, 71%); Mp. 247–249 °C, $[\alpha]_D^{20}$ +1765 (c 0.2, MeOH). HRMS calculated for $C_{31}H_{30}N_3NiO_3^+$: 550.1635; found: 550.1645. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.63 (s, 3H, CH_3), 1.93 (dd, 1H, *J* = 17.4, 2.6, $CH_2C{\equiv}CH$), 2.03–2.20 (m, 2H, γ-H Pro), 2.29 (t, 1H, *J* = 2.6, C≡CH), 2.42–2.67 (m, 2H, β-H Pro), 2.78 (dd, 1H, *J* = 17.4, 2.6, $CH_2C{\equiv}CH$), 3.40–3.50 (m, 1H, γ-CH_2 Pro), 3.45 (dd, 1H, *J* = 10.8, 5.6, α-CH Pro), 3.54 (d, 1H, *J* = 12.5,CH_2Ph), 3.69–3.71 (m, 1H, β-CH_2 Pro), 4.52 (d, 1H, *J* = 12.5, CH_2Ph), 6.61 (ddd, 1H, *J* = 8.4, 6.5, 1.0, 4-H Ph), 6.66 (ddd, 1H, *J* = 8.4, 1.9, 3-H Ph), 7.02 (d, 1H, *J* = 7.6, 2-H Ph), 7.09 (ddd, 1H, *J* = 8.6, 6.5, 1.9, 5-H Ph), 7.20 (d, 1H, *J* = 7.5, 4-H Ph), 7.33–7.42 (m, 3H, Ar), 7.48–7.54 (m, 2H, Ar), 7.81 (m, 1H, Ar), 8.92 (dd, 1H, *J* = 8.6, 1.0, 6-H Ph), 8.25–8.31(m, 2H, 2,2-H Ph). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 24.0 (γ-CH_2 Pro), 27.4 (CH_3), 30.9 (CH_2), 31.1 (CH_2), 58.1 (β-CH_2Pro), 64.0 (CH_2Ph), 70.4 (α-C, Pro), 72.4 (CCH_3), 79.8 (≡CH) 80.5 (C≡CH), 120.8 (CHAr), 124.3 (CHAr), 127.6 (CHAr), 127.8 (CHAr), 128.1 (CHAr), 128.5 (CHAr), 129.0 (CHAr), 129.1 (CHAr), 129.8 (CHAr), 131.6 (CHAr), 131.8 (CHAr), 133.3 (CHAr), 134.2 (CAr), 136.3 (CAr), 141.7 (CAr), 172.8 (C=N), 180.7 (COO), 181.3 (C=O).

2.3. General Procedure for Synthesis of Complexes **2a–f**

(*S*)-BPB-Ni-(*S*)-2-amino-2-methylpent-4-ynoic acid complex **1** (1.0 mmol, 1.0 equiv.) was added to a stirred solution of $Pd(PPh_3)_4$ (5 mol%), CuI (10 mol%), bromobenzene (1.2 equiv.), *i*Pr_2NH (3 mL) and 1,4-dioxane (4 mL). The mixture was stirred for 7 h at 75–80 °C. After cooling to room temperature, the mixture was diluted with DCM and washed two times

with water. The organic layer was dried over Na_2SO_4 and filtered. Afterwards, the solvent was removed under reduced pressure and the residue was purified using column chromatography (EtOAc/acetone 10:1) to obtain **2a**-**f** as a red solid.

(S)-BPB-Ni-(S)-2-amino-2-methyl-5-phenylpent-4-ynoic acid complex (**2a**). The reaction of **1** (600 mg, 1.1 mmol), $Pd(PPh_3)_4$ (62.4 mg, 0.05 mmol), CuI (20.9 mg, 0.11 mmol) and bromobenzene (0.14 mL, 1.31 mmol) in *i*Pr_2NH (3 mL) and 1,4-dioxane (4.5 mL) for 6 h gave a red solid (513 mg, 75%); Mp. 120–122 °C. $[\alpha]_D^{20}$ +1792.86 (c 0.07, MeOH). HRMS calculated for $C_{37}H_{33}N_3NiO_3^+$: 626.1948; found: 626.1957. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.40 (s, 3H, CH_3), 1.57–1.69 (m, 1H, γ-H Pro), 1.89–2.12 (m, 3H, β-$H_{a,b}$ and δ-H_a Pro), 2.30 (d, 1H, *J* = 17.0, CH_2), 3.01 (d, 1H, *J* = 17.0, CH_2), 2.93–3.10 (m, 1H, γ-H_b Pro), 3.31 (dd, 1H, *J* = 10.4, 6.2, α-H Pro), 3.65 (d, 1H, *J* = 12.6, CH_2Ph), 3.66–3.73 (m, 1H, δ-H_b Pro), 4.45 (d, 1H, *J* = 12.6, CH_2Ph), 6.62–6.69 (m, 2H, 3,4-H Ph), 7.16 (ddd, 1H, *J* = 8.4, 5.6, 3.0, 5-H Ph), 7.26–7.53 (m, 13H, Ar), 8.04–8.12 (m, 3H, 6-H Ph and CH_2Ph). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 23.3 (γ-CH_2), 29.1 (CH_3), 30.2 (β-CH_2), 31.1 (CH_2), 57.6 (δ-CH_2), 63.6 (CH_2Ph), 69.9 (α-CH), 76.3 (CCH_3), 85.5 ($CH_2C{\equiv}C$), 86.0 ($CH_2C{\equiv}C$), 120.7 (CHAr) 123.4 (CHAr), 124.3 (CHAr), 127.0 (CHAr), 128.0 (CHAr), 128.3 (CHAr), 128.4 (CHAr), 128.5 (CHAr), 128.53 (CHAr), 128.9 (CHAr), 129.0 (CHAr), 129.7 (CHAr), 130.1 (CHAr), 131.7 (CHAr), 131.8 (CHAr), 132.1 (CHAr), 133.5 (CHAr), 133.6 (CAr), 136.5 (CAr), 142.2 (CAr), 173.5 (C=N), 180.8 (COO), 182.2 (C=O).

(S)-BPB-Ni-(S)-2-amino-2-methyl-5-(p-tolyl)pent-4-ynoic acid complex (**2b**). The reaction of **1** (600 mg, 1.1 mmol), $Pd(PPh_3)_4$ (62.4 mg, 0.05 mmol), CuI (20.9 mg, 0.11 mmol) and 1-bromo-4-methylbenzene (224.7 mg, 1.31 mmol) in *i*Pr_2NH (3 mL) and 1,4-dioxane (4.5 mL) for 6 h gave a red solid (491 mg, 70%); Mp. 130–132 °C. $[\alpha]_D^{20}$ +936.67 (c 0.15, MeOH). HRMS calculated For $C_{38}H_{35}N_3NiO_3^+$: 640.2105; found: 640.2119. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.39 (s, 3H, CH_3), 1.59–1.70 (m, 1H, γ-H Pro), 1.93–2.13 (m, 3H, β-CH_2 and δ-H_a Pro), 2.29 (m, 1H, *J* = 17.2, $CH_2C{\equiv}C$), 2.34 (s, 3H, CH_3-Ar), 3.00 (d, 1H, *J* = 17.2, $CH_2C{\equiv}C$), 2.99–3.14 (m, 1H, γ-H_b Pro), 3.31 (dd, 1H, *J* = 10.4, 6.4, α-H Pro), 3.66 (d, 1H, *J* = 12.6, CH_2Ph), 3.70 (ddd, 1H, *J* = 11.0, 6.3, 2.5, δ-H_b Pro), 4.46 (d, 1H, *J* = 12.6, CH_2Ph), 6.62–6.69 (m, 2H, 3,4-H, Ph), 7.09–7.13 (m, 1H $PhCH_3$), 7.16 (ddd, 1H, *J* = 8.5, 5.5, 2.9, 5-H Ph), 7.26–7.32 (m, 1H, 4-Ph), 7.33–7.52 (m, 9H, Ar), 8.05–8.11 (m, 3H, 6-H Ph and 2-Ph). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 21.6 (CH_3-Ar), 23.4 (γ-CH_2), 29.2 (CH_3), 30.3 (β-CH_2), 31.2 (CH_2), 57.6 (δ-CH_2), 63.5 (CH_2Ph), 69.9 (α-CH), 76.3 (CCH_3), 85.3 ($CH_2C{\equiv}C$), 85.7 ($CH_2C{\equiv}C$), 120.4 (CHAr), 120.7 (CHAr), 124.3 (CHAr), 126.9 (CHAr), 128.0 (CHAr), 128.3 (CHAr), 128.5 (CHAr), 128.9 (CHAr), 129.0 (CHAr), 129.3 (CHAr), 129.7 (CHAr), 130.1 (CHAr), 131.7 (CHAr), 131.8 (CHAr), 132.0 (CHAr), 133.5 (CHAr), 133.6 (CHAr), 136.6 (CAr), 138.5 (CAr), 142.2 (CAr), 173.5 (C=N), 180.7 (COO), 182.3 (C=O).

(S)-BPB-Ni-(S)-2-amino-5-(4-fluorophenyl)-2-methylpent-4-ynoic acid complex (**2c**). The reaction of **1** (600 mg, 1.1 mmol), $Pd(PPh_3)_4$ (62.4 mg, 0.05 mmol), CuI (20.9 mg, 0.11 mmol) and 1-bromo-4-fluorobenzene (0.14 mL, 1.31 mmol) in *i*Pr_2NH (3 mL) and 1,4-dioxane (4.5 mL) for 6 h gave a red solid (531 mg, 75%); Mp. 120–122 °C. $[\alpha]_D^{20}$ +1068.75 (c 0.32, MeOH). HRMS calculated for $C_{37}H_{32}N_3FNiO_3^+$: 644.1854; found: 644.1857. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.39 (s, 3H, CH_3), 1.60–1.73 (m, 1H, γ-H_a Pro), 1.93–2.15 (m, 3H, β-$H_{a,b}$ and δ-H_a Pro), 2.30 (d, 1H, *J* = 17.2, CH_2), 3.00 (d, 1H, *J* = 17.2, CH_2), 2.96–3.12 (m, 1H, γ-H_b Pro), 3.33 (dd, 1H, *J* = 10.6, 6.1, α-H Pro), 3.64–3.71 (m, 1H, δ-H_b Pro), 3.67 (d, 1H, *J* = 12.6, CH_2Ph), 4.45 (d, 1H, *J* = 12.6, CH_2Ph), 6.62–6.69 (m, 2H, 3,4-H Ph), 6.97–7.05 (m, 2H, C_6H_4F), 7.17 (ddd, 1H, *J* = 8.5, 4.7, 3.9, 5-H Ph), 7.26–7.56 (m, 10H, Ar), 8.04–8.11 (m, 3H, 6-H, C_6H_4 and 2-Ph). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 23.4 (γ-CH_2), 29.1 (CH_3), 30.3 (β-CH_2), 31.1 (CH_2), 57.5 (δ-CH_2), 63.6 (CH_2Ph), 69.9 (α-CH), 76.2 (CCH_3), 84.4 ($CH_2C{\equiv}C$), 85.8 (d, J_{CF} = 1.8, $CH_2C{\equiv}C$), 115.9 (d, J_{CF} = 22.0, CFAr), 120.8 (CHAr), 124.3 (CHAr), 127.0 (CHAr), 127.9 (CHAr), 128.3 (CHAr), 128.4 (CHAr), 129.0 (CHAr), 129.1 (CHAr), 129.8 (CHAr), 130.1 (CHAr), 131.8 (CHAr), 131.8 (CHAr), 133.5 (CHAr), 133.6 (CHAr), 134.0 (d, J_{CF} = 8.4, CFAr), 136.5 (CAr), 138.5 (CAr), 142.1 (CAr), 162.6 (d, J_{CF} = 246.0, CFAr), 173.5 (C=N), 180.7 (COO), 182.2 (C=O).

(S)-BPB-Ni-(S)-2-amino-5-(4-bromophenyl)-2-methylpent-4-ynoic acid complex (**2d**). The reaction of **1** (600 mg, 1.1 mmol), $Pd(PPh_3)_4$ (62.4 mg, 0.05 mmol), CuI (20.9 mg, 0.11 mmol) and 1-bromo-4-iodobenzene (371.7 mg, 1.31 mmol) in *i*Pr_2NH (3 mL) and 1,4-dioxane (4.5 mL) for 6 h gave a red solid (525 mg, 68%); Mp. 123–125 °C. $[\alpha]_D^{20}$ +417.24 (c 0.29, MeOH). HRMS calculated For $C_{37}H_{32}N_3BrNiO_3^+$: 704.1053; found: 704.1065. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.38 (s, 3H, CH_3), 1.63–1.75 (m, 1H, γ-H_a Pro), 1.93–2.14 (m, 3H, β-CH_2, δ-H_a Pro), 2.30 (d, 1H, *J* = 17.2, $CH_2C{\equiv}C$), 3.00 (d, 1H, *J* = 17.2, $CH_2C{\equiv}C$), 2.96–3.12 (m, 1H, γ-H_b Pro), 3.35 (dd, 1H, *J* = 10.5, 6.1, α-H Pro), 3.63–3.69 (m, 1H, δ-H_b Pro), 3.67 (d, 1H, *J* = 12.6, CH_2Ph), 4.44 (d, 1H, *J* = 12.6, CH_2Ph), 6.60–6.70 (m 2H, 3,4-H Ph), 7.12–7.21 (m, 2H, C_6H_4F), 7.26–7.53 (m, 12H, Ar), 8.05 (br. d, 1H, *J* = 8.6, 6-H Ph), 8.07–8.12 (m, 2H, 2-Ph). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 23.4 (γ-CH_2 Pro), 29.1 (CH_3), 30.3 (β-CH_2), 31.1 (CH_2), 57.5 (δ-CH_2), 63.6 (CH_2Ph), 76.2 (CCH_3), 84.4 ($CH_2C{\equiv}C$), 87.4 ($CH_2C{\equiv}C$), 120.8 (CHAr), 122.3, 122.7 (Ar), 124.3 (CHAr), 127.1 (CHAr), 127.9 (CHAr), 128.3 (CHAr), 128.4 (Ar), 129.0 (2·CHAr), 129.1 (CHAr), 129.8 (CHAr), 130.1 (CHAr), 131.8 (2·CHAr), 131.8 (2·CHAr), 131.9 (CHAr), 133.5 (CHAr), 133.6 (CAr), 136.5 (CAr), 142.1 (CAr), 173.6 (C=N), 180.7 (COO), 182.1 (C=O).

(S)-BPB-Ni-(S)-2-amino-5-(2,4-difluorophenyl)-2-methylpent-4-ynoic acid complex (**2e**). The reaction of **1** (600 mg, 1.1 mmol), $Pd(PPh_3)_4$ (62.4 mg, 0.05 mmol), CuI (20.9 mg, 0.11 mmol) and 1-bromo-2,4-difluorobenzene (0.15 mL, 1.31 mmol) in *i*Pr_2NH (3 mL) and 1,4-dioxane (4.5 mL) for 6 h gave a red solid (507 mg, 70%); Mp. 118–120 °C. $[\alpha]_D^{20}$ +1061.67 (c 0.30, MeOH). HRMS calculated for $C_{37}H_{31}N_3F_2NiO_3^+$: 662.1760; found: 662.1771. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.43 (s, 3H, CH_3), 1.69–1.80 (m, 1H, γ-H_a Pro), 1.94–2.10 (m, 2H, β-H_a and δ-H_a Pro), 2.12–2.20 (m, 2H, β-H_β Pro), 2.23 (d, 1H, *J* = 17.2, $CH_2C{\equiv}C$), 3.02 (d, 1H, *J* = 17.2, $CH_2C{\equiv}C$), 3.04–3.20 (m, 1H, γ-H_b Pro), 3.34 (dd, 1H, *J* = 10.6, 6.1, α-H Pro), 3.66–3.73 (m, 1H, δ-H_b Pro), 3.69 (d, 1H, *J* = 12.6, CH_2Ph), 4.46 (d, 1H, *J* = 12.6, CH_2Ph), 6.62–6.68 (m, 2H, 3,4-H Ph), 6.82–6.89 (m, 2H, Ar), 7.17 (ddd, 1H, *J* = 8.4, 5.0, 3.6, 5-H Ph), 7.27–7.36 (m, 2H, Ar), 7.39–7.54 (m, 7H, Ar), 8.03–8.11 (m, 3H, 6-H Ph and 2-Ph). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 23.4 (γ-CH_2), 29.2 (CH_3), 30.4 (β-CH_2 Pro), 31.0 (CH_2), 57.5 (δ-CH_2 Pro), 63.6 (CH_2Ph), 70.0 (α-CH Pro), 75.4, 76.1 (CCH_3), 76.6 ($CH_2C{\equiv}C$), 91.4 (dd, $J_{C,F}$ = 4.3, 1.8, $CH_2C{\equiv}C$), 104.5 (t, $J_{C,F}$ = 25.3, 3-CH, $C_6H_3F_2$), 111.7 (dd, J_{CF} = 21.9, 4.0, 5-CH, CFAr), 120.8 (4-CH, Ar), 124.4 (6-CH, Ar), 127.0 (CHAr), 128.07 (CHAr), 128.3 (CHAr), 128.5, 129.0 (2·CHAr), 129.1 (CHAr), 129.7 (CHAr), 129.8 (CHAr), 130.1 (CHAr), 131.8 (CHAr), 131.8 (2·CHAr), 133.5 (CHAr), 133.6 (CAr), 134.6 (dd, J_{CF} = 9.7, 2.7, 1C, CFAr), 136.5 (CAr), 142.1 (CAr), 162.7 (dd, J_{CF} = 252.2, 11.5 CFAr), 163.7 (dd, J_{CF} = 254.2, 12.0, CFAr), 173.5 (C=N), 180.7 (COO), 182.1 (C=O).

(S)-BPB-Ni-(S)-2-amino-5-(3,4-difluorophenyl)-2-methylpent-4-ynoic acid complex (**2f**). The reaction of **1** (600 mg, 1.1 mmol), $Pd(PPh_3)_4$ (62.4 mg, 0.05 mmol), CuI (20.9 mg, 0.11 mmol) and 4-bromo-1,2-difluorobenzene (0.15 mL, 1.31 mmol) in *i*Pr_2NH (3 mL) and 1,4-dioxane (4.5 mL) for 6 h gave a red solid (457 mg, 63%); Mp. 118–120 °C. $[\alpha]_D^{20}$ +1297.65 (c 0.09, MeOH). HRMS calculated For $C_{37}H_{31}N_3F_2NiO_3^+$: 662.1760; found: 662.1769. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.38 (s, 3H, CH_3), 1.69–1.81 (m, 1H, γ-H_a Pro), 1.95–2.22 (m, 3H, β-CH_2, δ-H_a Pro), 2.30 (d, 1H, *J* = 17.2, $CH_2C{\equiv}C$), 2.99 (d, 1H, *J* = 17.2, $CH_2C{\equiv}C$), 2.99–3.15 (m, 1H, γ-H_b Pro), 3.37 (dd, 1H, *J* = 10.5, 6.1, α-H Pro), 3.65–3.72 (m, 1H, δ-H_b Pro), 3.69 (d, 1H, *J* = 12.6, CH_2Ph), 4.44 (d, 1H, *J* = 12.6, CH_2Ph), 6.63–6.70 (m, 2H, 3,4-H Ph), 7.05–7.24 (m, 3H, Ar), 7.28–7.37 (m, 4H, Ar), 7.39–7.48 (m, 3H, Ar), 7.49–7.54 (m, 2H, Ar), 8.05–8.11 (m, 3H, Ar). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 23.3 (γ-CH_2), 29.1(CH_3), 30.4 (β-CH_2 Pro), 31.0 (CH_2), 57.5 (δ-CH_2 Pro), 63.7 (CH_2Ph), 70.0 (α-CH Pro), 76.1 (CCH_3), 83.2 (t, J_{CF} = 2.3, $CH_2C{\equiv}C$), 86.8 (d, J_{CF} = 2.0, $CH_2C{\equiv}C$), 117.8 (d, J_{CF} = 17.6, CFAr), 120.2 (dd, J_{CF} = 7.6, 4.2, 1-C, CFAr), 120.8 (d, J_{CF} = 18.4, 4-CH, Ar), 120.9 (CHAr), 124.3 (CHAr), 127.1 (CHAr), 127.8 (CHAr), 128.3 (CHAr), 128.3 (CHAr), 128.8 (dd, J_{CF} = 6.4, 3.5, Ar), 129.0 (CHAr), 129.1 (CHAr), 129.8 (CHAr), 130.0 (CHAr), 131.8 (CHAr), 131.9 (CHAr), 133.5 (CHAr), 133.6 (CAr), 136.5 (CAr), 142.1 (CAr), 150.1 (dd, J_{CF} = 249.6, 12.8, CFAr), 150.7 (dd, J_{CF} = 251.6, 12.3 CFAr), 173.6 (C=N), 180.7 (COO), 182.1 (C=O).

2.4. General Procedure for the Isolation of AAs **3a–f**

Starting from the corresponding complexes (*S*,*S*)-**2a–f** (0.7–0.9 mmol) respectively, which were dissolved in 20–30 mL of MeOH, 10 mL of water as well as 2 mL of 12 M HCl were added. The mixture was heated to 60 °C and stirred for 30 min (the color changed from red to yellow-green). After cooling to room temperature, the mixture was diluted with water and extracted with $CHCl_3$ (4 times) to remove (*S*)-**BPB** ligand. Notably, the obtained unnatural AAs **3** directly precipitate from the reaction mixture after decomposition due to the hydrophobicity of the alkyne group, which makes the process more practical and useful, avoiding the ion-exchange column to obtain **3a–f**.

(S)-2-amino-2-methyl-5-phenylpent-4-ynoic acid (**3a**). The decomposition of **2a** (486 mg, 0.78 mmol) gave **3a** as a white solid (147 mg, 94%). Mp. 282–284 °C. $[\alpha]_D^{20}$ –5.12 (c 0.078, MeOH). HRMS calculated for $C_{12}H_{13}NO_2^+$: 204.1019; found: 204.1026. ^{1}H NMR (300 MHz, DMSO-d6+CCl_4+CF_3COOD): δ = 1.60 (s, 3H, CH_3), 2.99 (d, 1H, J = 17.3, CH_2), 3.02 (d, 1H, J = 17.3, CH_2), 7.28–7.33 (m, 3H), 7.41–7.47 (m, 2H, Ph), 8.70 (br. s, 2H, NH_2), 10.20 (COOH). ^{13}C NMR (75.5 MHz, DMSO+CCl_4+CF_3COOD): δ = 21.2 (CH_3), 27.7 (CH_2), 58.0 (CCH_3), 82.4 ($CH_2C{\equiv}C$), 84.3 ($CH_2C{\equiv}C$), 122.5 (C_{ipso}), 127.8 (CHAr), 127.9 (CHAr), 131.4 (CHAr), 171.2 (COOH).

(S)-2-amino-2-methyl-5-(p-tolyl)pent-4-ynoic acid (**3b**). The decomposition of **2b** (477 mg, 0.74 mmol) gave **3b** as a white solid (147 mg, 91%). Mp. 282–284 °C. $[\alpha]_D^{20}$ –3.80 (c 0.105, MeOH). HRMS calculated For $C_{13}H_{15}NO_2^+$: 218.1176; found: 218.1182. ^{1}H NMR (300 MHz, D_2O+CF_3COOD): δ = 1.66 (s, 3H, CH_3), 2.28 (s, 3H, $PhCH_3$), 2.99 (d, 1H, J = 17.7, CH_2), 3.14 (d, 1H, J = 17.3, CH_2), 7.14–7.20 (m, 2H, Ph), 7.31–7.37 (m, 2H, C_6H_4). ^{13}C NMR (75.5 MHz, D_2O+CF_3COOD): δ = 19.8 (CH_3-Ar), 20.3 (CH_3), 27.0 (CH_2), 58.6 (CCH_3), 80.0 ($CH_2C{\equiv}C$), 84.7 ($CH_2C{\equiv}C$), 117.7 (CHAr), 120.5 (CHAr), 131.0 (CHAr), 139.1 (CAr), 172.0 (COOH).

(S)-2-amino-5-(4-fluorophenyl)-2-methylpent-4-ynoic acid (**3c**). The decomposition of **2c** (531 mg, 0.82 mmol) gave **3c** as a white solid (174 mg, 96%). Mp. 280–282 °C. $[\alpha]_D^{20}$ –6.05 (c 0.09, MeOH). HRMS calculated for $C_{12}H_{12}FNO_2^+$: 222.0925; found: 222.0934. ^{1}H NMR (300 MHz DMSO-d6+CF_3COOD): δ = 1.63 (s, 3H, CH_3), 3.01 (d, 1H, J = 17.2, CH_2), 3.08 (d, 1H, J = 17.2, CH_2), 6.69–7.07 (m, 2H, C_6H_4F), 7.47–7.53 (m, 2H, C_6H_4F). ^{13}C NMR (75.5 MHz, DMSO+CF_3COOD): δ = 21.1 (CH_3), 27.6 (CH_2), 58.1 (CCH_3), 82.4 ($CH_2C{\equiv}C$), 83.2 ($CH_2C{\equiv}C$), 115.1 (d, J_{CF} = 21.9, CHAr), 133.62 (d, J_{CF} = 8.2, CHAr), 161.8 (d, J_{CF} = 248.6, CFAr), 171.0 (COOH).

(S)-2-amino-5-(4-bromophenyl)-2-methylpent-4-ynoic acid (**3d**). The decomposition of **2d** (525 mg, 0.74 mmol) gave **3d** as a white solid (150 mg, 71%). Mp. 283–285 °C. $[\alpha]_D^{20}$ –4.80 (c 0.095, MeOH). HRMS calculated for $C_{12}H_{12}BrNO_2^+$: 282.0124; found: 282.0133. ^{1}H NMR (300 MHz, D_2O+CF_3COOD): δ = 1.69 (s, 3H, CH_3), 3.04 (d, 1H, J = 17.7, CH_2), 3.18 (d, 1H, J = 17.7, CH_2), 7.35 (d, J = 8.5, 2H, C_6H_4Br), 7.53 (d, J = 8.5, 2H, C_6H_4Br). ^{13}C NMR (75.5 MHz, D_2O+CF_3COOD): δ = 20.4 (CH_3), 27.0 (CH_2), 58.5 (CCH_3), 81.8 ($CH_2C{\equiv}C$), 83.7 ($CH_2C{\equiv}C$), 120.0 (CHAr), 121,9 (CHAr), 130.9 (CHAr), 132.6 (CHAr), 172.0 (COOH).

(S)-2-amino-5-(2,4-difluorophenyl)-2-methylpent-4-ynoic acid (**3e**). The decomposition of **2e** (507 mg, 0.77 mmol) gave **3e** as a white solid (180 mg, 95%). Mp. 284–286 °C. $[\alpha]_D^{20}$ –6.67 (c 0.12, MeOH). HRMS calculated for $C_{12}H_{11}F_2NO_2^+$: 240.0831; found: 240.0842. ^{1}H NMR (300 MHz, DMSO-d6+CF_3COOD): δ = 1.65 (s, 3H, CH_3), 3.02 (d, 1H, J = 17.7, CH_2), 3.16 (d, 1H, J = 17.7, CH_2), 7,01–7.63 (m, 1H, C_6H_3). ^{13}C NMR (75 MHz, DMSO): δ = 21.0 (CH_3), 27.6 (CH_2), 57.8 (CCH_3), 76.6 (C≡C), 87.5 ($CH_2C{\equiv}$), 103.6 (t, J_{CF} = 25.3 Hz, CHAr), 107.3 (dd, J_{CF} = 15.9, 4.1 Hz, CHAr), 111.2 (dd, J_{CF} = 21.8, 3.2 Hz, CHAr), 134.9 (dd, J_{CF} = 9.6, 2.3 Hz, CAr), 161.9 (dd, J_{CF} = 251.4, 11.4 Hz, CFAr), 162.4 (dd, J_{CF} = 253.3, 12.2 Hz, CFAr), 171.0 (COOH).

(S)-2-amino-5-(3,4-difluorophenyl)-2-methylpent-4-ynoic acid (**3f**). The decomposition of **2f** (501 mg, 0.76 mmol) gave **3f** as a white solid (129 mg, 72%). Mp. 278–280 °C. $[\alpha]_D^{20}$ –7.2 (c 0.10, MeOH). HRMS calculated For $C_{12}H_{11}F_2NO_2^+$: 240.0831; found: 240.0844. ^{1}H NMR (300 MHz, D_2O+CF_3COOD): δ = 1.67 (s, 3H, CH_3), 3.02 (d, 1H, J = 17.8, CH_2), 3.18 (d, 1H, J = 17.8, CH_2), 7,15–7.26 (m, 2H), 7.28–7.37 (m, 1H, C_6H_3). ^{13}C NMR (75.5 MHz,

D_2O+CF_3COOD): δ = 20.4 (CH_3), 26.8 (CH_2), 58.5 (CCH_3), 81.1 ($CH_2C{\equiv}C$), 82.6 ($CH_2C{\equiv}C$), 116.8 (d, J_{CF} = 17.9, CHAr), 117.9 (dd, J_{CF} = 8.2, 4.0, CHAr), 119.8 (d, J_{CF} = 18.8, CHAr), 128.1 (dd, J_{CF} = 6.8, 3.5, CFAr), 148.8 (dd, J_{CF} = 246.2, 13.1, CFAr), 149.8 (dd, J_{CF} = 249.3, 12.5, CFAr), 171.8 (COOH).

2.5. Synthesis of N-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-(S)-2-methyl-5-phenylpent-4 -ynoic acid **4**

(*S*)-2-amino-2-methyl-5-phenylpent-4-ynoic acid **3a** (500 mg, 4.34 mmol) was suspended in a 10% water solution of Na_2CO_3 (1.5 g, 80 mL) and Fmoc-OSu (1.43 g, 4.25 mmol) dissolved in 3 mL of dioxane was added under stirring. The mixture was stirred at room temperature overnight. TLC (eluent: $CHCl_3$/EtOAc/CH_3OH, 4:2:1) showed that the reaction was complete. Water was added to the reaction mixture and extracted with diethyl ether (2 × 5 mL) to remove traces of Fmoc-OSu, and then the reaction mixture was acidified using 2 M HCl to pH = 2~3. Then, the mixture was extracted with EtOAc (3 × 25 mL), which was washed with water, dried over Na_2SO_4 and evaporated.

Mp. 173–175 °C. HRMS calculated for $C_{27}H_{23}NO_4^+$: 426.1700; found: 426.1715. ^{1}H NMR (300 MHz, DMSO-d6): δ = 1.58 (s, 3H), 2.95 (d, 1H, *J* = 17.0, $CH_2C{\equiv}$), 3.21 (d, 1H, *J* = 17.0, $CH_2C{\equiv}$), 4.36–4.19 (m, 3H, OCH_2 and CH_2CH), 7.45–7.18 (m, 10H, Ar), 7.68 (d, 1H, *J* = 7.5, Ar), 7.74 (d, 1H, *J* = 7.5, Ar). ^{13}C NMR (75 MHz, DMSO): δ = 22.7 (CH_3), 26.8 ($CH_2C{\equiv}$), 46.6 (CH_2CH), 57.6 (CH_2CH), 65.5 (CCH_3), 82.3 (≡CPh), 86.1 (C≡CPh), 119.3 (CHAr), 123.3 (CAr), 124.9, 126.56, 127.0, 127.1, 127.7, 131.1 (CHAr), 140.5, 143.6 (CAr), 154.3 (C=O), 174.0 (COOH).

2.6. Synthesis of N-9-fluorenylmethyloxycarbonyl-(S)-2-amino-2-methyl-5-phenylpent-4-ynoic Acid Succinimide Ester **5**

An amount of 113 mg (0.55 mmol) of dicyclohexylcarbodiimide (DCC), dissolved in 3 mL of dioxane, was added at 0 °C to 212 mg (0.5 mmol) of **4** and 63.3 mg (1.1 mmol) of *N*-hydroxysuccinimide in a mixture of 4 mL of 1,4-dioxane and 2 mL of CH_2Cl_2. The reaction mixture was stirred for ~2 h at 0 °C and left overnight in a refrigerator. The analysis was performed using TLC (SiO_2, eluent: $CHCl_3$/EtOAc/CH_3OH, 4:2:1, chlorotoluidine as a detector). The precipitate was filtered off, the solvent removed on a rotary evaporator, and the solid crystallized using a mixture of EtOAc/hexane (1:2). Yield: 236 mg (91%). The compound was directly used in the next step without characterization.

2.7. Synthesis of Dipeptide N-9-fluorenylmethyloxycarbonyl-α-phenyl-(S)-propargylalanylglycine **6**

Into a round-bottom flask with a magnetic stirring bar, 0.18 g (0.66 mmol) of glycine, 1.25 mL (0.63 mmol) of 0.5M NaOH solution and 0.016 g (0.19 mmol) of baking soda were placed. Next, 0.24 g (0.6 mmol) of succinimide ester **5** in 2 mL of 1,4-dioxane was added to the mixture at room temperature, and the reaction mixture was stirred for 3 h. The next day, 5 mL of EtOAc and 1.45 mL of 10% citric acid were added to the flask. After vigorous stirring, the organic layer was separated, and the aqueous layer was extracted with EtOAc (2 × 5 mL). The organic layer was dried over anhydrous Na_2SO_4, and then the solvent was evaporated to dryness resulting in the desired product with an 87% yield.

Mp. 98–100 °C. $[\alpha]_D^{20}$ –81.4 (c 1.0, MeOH). HRMS calculated for $C_{29}H_{26}N_2O_5^+$: 515.1635; found: 515.1645**.** ^{1}H NMR (300 MHz, DMSO-d6): δ = 1.56 (s, 1H, CH_3), 2.97 (d, 1H, *J* = 17.9, $CH_2C{\equiv}$), 3.25 (d, 1H, *J* = 17.9, $CH_2C{\equiv}$), 3.74 (dd, 1H, *J* = 17.7, 5.4, $NHCH_2$), 3.85 (dd, 1H, *J* = 17.7, 5.4, $NHCH_2$), 4.33–4.21 (m, 3H, $CHCH_2O$), 7.40–7.16 (m, 10H, CHAr and 2NH), 7.76–7.61 (m, 5H, CHAr). ^{13}C NMR (75 MHz, DMSO): δ = 24.5 (CH_3), 46.7 (CH_2), 58.1 (CH_2), 72.2 (CCH_3), 82.3 (C≡C), 86.4 ($CH_2C{\equiv}$), 119.3 (CHAr), 123.5, 125.0, 126.5, 127.0, 127.1, 127.7, 131.2, 140.6 (CAr), 143.7, 154.3, 170.9 (C=O), 172.7, 174.4 (COOH).

2.8. Determination of Collagenase Activity

Collagenase G from *Clostridium (Hathewaya) histolyticum* (EC 3.4.24.3) and miscellaneous reagents were purchased from Sigma-Aldrich (now Merck). Collagenase activity was determined using a known method [45]. The reaction mixture contained 10 mg/mL gelatin,

0.05 M HEPES buffer, pH = 7.2, and 0.025 mg/mL collagenase (activated by 0.1 M $CaCl_2$). An aliquot (50 µL) was taken and the remaining mixture was incubated at 37 °C. Aliquots were taken every 30 min. The reaction in the aliquots was stopped by addition of 6 µL of 30% trichloroacetic acid. The concentration of free amino groups in the reaction mixture was determined using an *ortho*-phthalaldehyde (OPA) reagent containing 0.2 M borate buffer, pH = 9.7, 1.18 mM mercaptoethanol and 0.167 mg/mL OPA. The aliquots of the reaction mixture were added to the OPA reagent (1.5 mL) and diluted with H_2O (1.5 mL). A340 was recorded after 7 min of incubation at 25 °C.

3. Results and Discussion

3.1. Syntheses of Complexes **2**, *AAs* **3** *and Dipeptide* **6**

The Ni(II) complex (*S*)-BPB-Ni-Ala was synthesized according to a published procedure [46] starting from commercially available alanine, Belokon's chiral auxiliary (*S*)-**BPB** and $Ni(NO_3)_2$ in a high yield (95%) (Scheme 1). The subsequent alkylation of alanine Ni(II) complexes with propargyl bromide provided the desired (*S*)-BPB-Ni-(*S*)-propargylalanine complex (*S*,*S*)-**1** in good yield (71%) with high diastereoselectivity (*dr* >20:1, the S_N2 reaction proceeded stereoselectively due to kinetic as well as thermodynamic control by a chiral auxiliary) (Scheme 1) [47]. Then, the functionalization of **1** via a Sonogashira reaction [48] delivered a number of diastereomerically pure Ni(II) complexes (*S*,*S*)-**2** featuring a propargyl alanine moiety (see Table 1). Sonogashira cross-coupling reactions were performed under optimized conditions [48] with a slight revision of reaction temperature. The reactions catalyzed by $Pd(PPh_3)_4$ and CuI were carried out in 1,4-dioxane in the presence of di-isopropyl amine at 80 °C under an argon atmosphere (Table 1).

Further, we investigated the scope of the reaction (Table 1). Electron-rich as well as electron-poor aryl bromides were tested and both delivered the desired products with good yields (63–75%). Di-fluoro-substituted aryl bromides such as 4-bromo-1,3-difluorobenzene (**f**) and 1-bromo-2,4-difluorobenzene (**e**) led to slightly lower yields, from which it can be assumed that both the spatial and electronic effects of functional groups play essential roles.

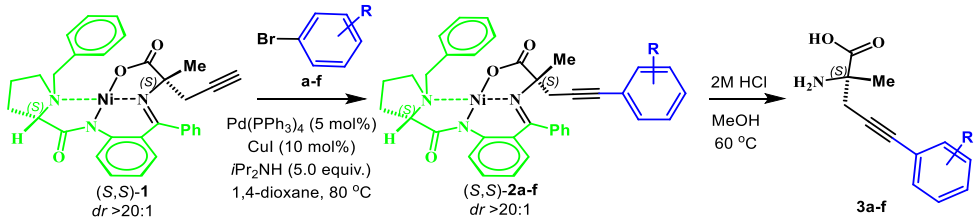
Next, the target AAs **3** were isolated from the corresponding complexes **2a-f** using a previously developed standard method [18] via an acidic decomposition in a refluxing mixture of 2 M HCl/MeOH with 71–96% yields, respectively (Table 1). Notably, the important advantage in this case is that the obtained unnatural α-AAs **3** directly precipitate from the reaction mixture after decomposition due to the hydrophobicity of the alkyne group, which makes the process more practical and useful, avoiding the ion-exchange column [15]. It is well known that the decomposition of the chiral Ni(II) complexes under acidic conditions provides α-AAs without racemization (see HPLC traces in the Supplementary Material, Figures S15–S20) [15–22]. Importantly, the chiral auxiliary ligand can be isolated from the resulting reaction mixture using filtration and/or extraction in an enantiopure form and is reusable for the synthesis of a new portion of the alanine Ni(II) complex [15–22].

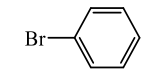
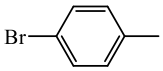
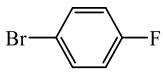
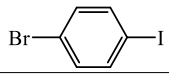
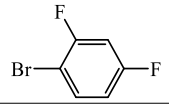
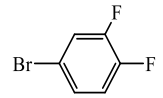
Then, we turned our attention to the synthesis of dipeptide based on the α,α-disubstituted unnatural α-AAs **3**. First, we focused on the condensation of unprotected α-AA **3a** with *N*-(Boc) and *C*-terminus (-OSu)-protected proteinogenic α-AAs such as glycine and alanine. Unfortunately, the reactions did not proceed, although we screened different conditions. In particular, we tested different solvents (1,4-dioxane, DMSO, DCM, etc.) with different base-to-base ratios (NaOH), conducting reactions at a temperature range = 20–45 °C.

Another approach was based on obtaining *N*- and *O*-protected AA **5** followed by subsequent coupling with free glycine to yield the desired dipeptide **6** (Scheme 2). First, an attempt to protect the amino group with a *tert*-butoxycarbonyl (Boc) group was unsuccessful. Then, we investigated the reaction of the introduction of a fluorenylmethoxycarbonyl (Fmoc) protecting group. To our delight, the desired *N*-protected derivative **4** was obtained in a quantitative yield. Further, the conversion of the resulting *N*-Fmoc-(*S*)-2-amino-2-methyl-5-phenylpent-4-ynoic acid **4** to succinimide ester was performed in the presence of dicyclohexylcarbodiimide (DCC), delivering product **5** (Scheme 2) [49,50]. Finally, the

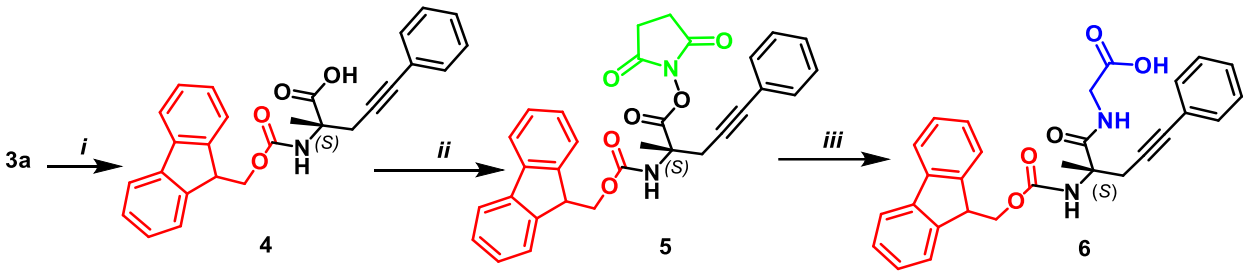
coupling of protected derivative **5** with glycine in the presence of NaOH led to dipeptide **6** containing both proteinogenic and non-proteinogenic α-AAs.

Table 1. Substrate scope of the Sonogashira cross-coupling reaction of complex **1** with various aryl bromides and the isolation of α-AAs **3** [a].



Aryl Bromide	Product	Yield, % [b]
	complex **2a**	75
	AA **3a**	94
	complex **2b**	70
	AA **3b**	91
	complex **2c**	75
	AA **3c**	96
	complex **2d**	68
	AA **3d**	71
	complex **2e**	69
	AA **3e**	95
	complex **2f**	63
	AA **3f**	72

[a] Reaction conditions: Ni(II) complex **1** (0.2 mmol, 1.0 equiv.), ArBr (1.2 equiv.), $Pd(PPh_3)_4$ (5 mol%), CuI (10 mol%), iPr_2NH (5.0 equiv.), 1,4-dioxane (1 mL), 80 °C, 6 h. Isolation of AAs **3a–f**: Ni(II) complex **2a–f** (0.5–1.1 mmol), MeOH (20–30 mL), 2M HCl (10 mL), 60 °C, 0.5 h. [b] The yields of complexes **2** were determined after column chromatography. The yields of AAs **3** were provided after crystallization.



Scheme 2. Synthesis of dipeptide **6**: (*i*) **3a** (100 mg, 0.5 mmol), 10% aq. Na_2CO_3 (68.9 mg, 0.65 mmol) and Fmoc-Osu (250 mg, 0.75 mmol), 1,4-dioxane (3 mL), RT, 0.5 h, 99% yield. (*ii*) **4** (212 mg, 0.5 mmol), HOSu (63.3 mg, 0.55 mmol), DCC (113 mg, 0.55 mmol), 6 mL 1,4-dioxane/CH_2Cl_2 (2:1), RT, 3 h, 91% yield. (*iii*) **5** (209 mg, 0.4 mmol), glycine (36 mg, 0.48 mmol), 0.5 M aq. NaOH (16 mg, 0.4 mmol), 4 mL 1,4-dioxane, RT, 3 h, 87% yield.

3.2. Biological Tests

The bacterial collagenolytic proteases are of increasing interest due to their virulent role in some diseases and their essential role in global nitrogen cycling [51]. The inhibition of these enzymes is appealing, as it does not attack the pathogen directly but rather blocks the colonization and infiltration of the host by the clostridia. Targeting extracellular enzymes

provides an essential benefit because the inhibitors do not need to cross the bacterial cell wall, which has turned out to be challenging in many cases [52]. It is known from the literature that non-proteinogenic AAs with similar structure (in particular, Mycosporine-like AAs from marine sources) show collagenase inhibition potential [53].

Ligand free energy was minimized using an MM2 force field and the truncated Newton–Raphson method. A crystallographic structure of collagenase G was modelled using PDB-ID 2Y50 [54]. The docking of ligand to enzyme was performed using AutoDock Vina software [55]. AutoDock uses the Lamarckian genetic algorithm by alternating local search with selection and crossover [56]. The ligands were ranked using an energy-based scoring function and a grid-based protein–ligand interaction was applied to speed up the score calculation.

All obtained compounds were tested on their ability to affect collagenase activity. The activity of collagenase was determined in the presence of the investigated non-proteinogenic α-AAs **3**. Compounds were added in a concentration range of 0.4–4.0 mM. According to the obtained data, collagenase was inhibited by all of the listed compounds (Table 2).

Table 2. Molecular docking data and inhibition results.

Run	Compound	ΔG (kcal/mol)	IC50 (mM)
1	**3a**	−6.2	1.25
2	**3b**	−5.9	0.93
3	**3c**	−6.0	1.45
4	**3d**	−6.6	1.14
5	**3e**	−6.7	2.57
6	**3f**	−6.1	0.59
7	**6**	−9.2	-

3.3. Molecular Docking

According to the molecular docking analysis, all studied structures show an ability to interact with collagenase. For all compounds, Gibbs free energy value was negative (Table 2; for details, see data in the Supplementary Material, Figures S21–S27). Of the investigated compounds, only two show the ability to interact very close to the active center (active and binding sites): α-AA **3f**, by its carbonyl oxygen atom of the carboxylic group, forms a hydrogen bond with the free amino group of Arg443 (2.055 Å) and **3b** forms only hydrophobic bonds, but too close to the active site [57]. The rest of the investigated compounds interact with different parts of the activator domain which is required for full activity on collagen [54]. AA **3a** forms a hydrogen bond with Ala252 (2.079 Å), the aromatic ring of **3d** forms a π-π interaction with Tyr201 (4.817 Å), **3e**, by its amino group, forms a H-bond with the carboxylic group of Gly250 (2.035 Å) and π-π with Tyr201 (4.451 Å), the carbonyl oxygen atom of the carboxyl group of **3c** and amino group of Gln215 (2.176 Å) and π-π Tyr201 (3.802 Å). According to our experimental data, compounds **3b** and **3f** showed the highest inhibitory activity with IC50 values of 0.93 mM and 0.59 mM, respectively. At the same time, according to the results of the docking analysis, these AAs did not show the highest level of interaction, but they were the only ones that interacted with the catalytic domain of collagenase and very close to the active site. The rest of the compounds interacted with enzymes at the activator subdomain which was required for full activity on collagen.

4. Conclusions

In summary, we elaborated an asymmetric metal-templated synthesis of enantiomerically pure (*S*)-2-amino-2-methyl-5-arylpent-4-ynoic acids **3** starting from a readily available chiral Ni(II) complex based on a chiral auxiliary (*S*)-**BPB** and Ala. The subsequent propargylation and arylation by the Sonogashira cross-coupling reaction using various aryl halides provided a series of diastereomerically pure complexes **2** with a triple bond in a side chain

with good yields. An acidic decomposition of the obtained complexes delivers to the target complex α-AAs **3**. Next, the Fmoc-protected α-AA and dipeptide **6** were synthesized. The bioactivity examination of the obtained α-AAs **3** showed that they inhibited the bacterial collagenolytic protease. Molecular docking analysis of all studied structures demonstrated an ability to interact with collagenase via a hydrogen bonding and π-π interactions. As a perspective, the obtained unnatural α-AAs **3** can be loaded onto the resin(s) for SPPS in order to produce modified peptides.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/sym15101924/s1, Figures S1–S27 contain NMR spectra, HPLC traces and molecular docking pictures.

Author Contributions: Conceptualization, Z.Z.M. and V.A.L.; methodology, H.I.H. and S.M.J.; formal analysis, Y.M.D., V.A.L., V.I.M. and Z.Z.M.; investigation, H.I.H., S.M.J. and A.S.S. (Armen S. Sargsyan); writing—original draft preparation, Z.Z.M.; writing—review and editing, V.A.L., V.I.M. and A.S.S. (Ashot S. Saghyan); project administration, Z.Z.M. and A.S.S. (Ashot S. Saghyan); funding acquisition, A.S.S. (Ashot S. Saghyan), V.A.L. and V.I.M. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: The data presented in this study are available in the article and Supplementary Material.

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Conflicts of Interest: The authors declare no conflict of interest.

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