RSC Advances

PAPER

View Article Online
View Journal | View Issue

Cite this: *RSC Adv.*, 2025, **15**, 35379

A^3-Mannich coupling reaction *via* chiral propargylglycine Ni(II) complex: an approach for synthesizing enantiomerically enriched unnatural α-amino acids

Liana Hayriyan,[ab] Anna Grigoryan,[ab] Hasmik Gevorgyan,[b] Avetis Tsaturyan,[ab] Armen Sargsyan,[b] Peter Langer,*[c] Ashot Saghyan*[ab] and Anna Mkrtchyan*[ab]

A novel hybrid synthetic approach was developed for the enantioselective synthesis of unnatural α-amino acids, utilizing chiral square-planar Ni(II) Schiff base complexes in combination with an A^3-coupling (Mannich-type) reaction. The methodology enabled the generation of a series of α-amino acid derivatives with excellent enantiomeric excess (≥99% ee) and high chemical yields under optimized reaction conditions involving CuI/$FeCl_3$ catalysis in toluene under argon. The reaction demonstrated strong dependence on the nature of the amine, with cyclic secondary amines yielding superior results compared to linear or primary analogs. The obtained products were structurally confirmed *via* X-ray crystallography and HPLC analysis, verifying the retention of stereochemical integrity. Molecular docking studies against collagenase indicated that all synthesized compounds could interact with the enzyme *via* various binding domains and interaction types, particularly hydrogen bonding. Subsequent *in vitro* collagenase inhibition assays revealed that all compounds, except **4a**, exhibited inhibitory activity, with compound **4e** demonstrating the highest potency (IC_{50} = 0.75 mM), despite not having the most favorable docking score. This highlights the importance of complementary *in silico* and experimental evaluations for reliable biological profiling. The presented strategy provides a versatile platform for the synthesis of structurally diverse, enantiomerically pure non-proteinogenic amino acids with promising bioactivity, offering valuable prospects for drug discovery and enzymatic inhibition studies.

Received 26th June 2025
Accepted 17th September 2025

DOI: 10.1039/d5ra04554a

rsc.li/rsc-advances

Introduction

A key aim and challenge in chemistry is designing reactions that create diverse, functional, optically active molecules from readily available, simple materials, with a high asymmetric yield. This is why, over the past twenty years, the synthesis of enantiomerically enriched molecules has become a central focus in organic synthesis.[1–3] Within this field, non-proteinogenic (unnatural) amino acids hold a particularly important position. In efforts to optimize physicochemical properties and enhance target selectivity, unnatural amino acids (UAAs) have emerged as indispensable building blocks in the design of peptide- and peptidomimetic-based therapeutics.[4] In addition to serving as key structural motifs in peptidomimetics, many UAAs also possess inherent biological activities. For instance, certain UAAs act as enzyme inhibitors,[5] while others demonstrate antimicrobial effects[6–8] or antiproliferative properties. These findings highlight the dual significance of UAAs, both as versatile intermediates in drug design and as bioactive compounds in their own right. In line with this, the search for innovative therapeutic strategies increasingly extends beyond natural molecules to include synthetic systems, such as amino acid–based derivatives, oligonucleotidic analogues, and hybrid structures.[9–13]

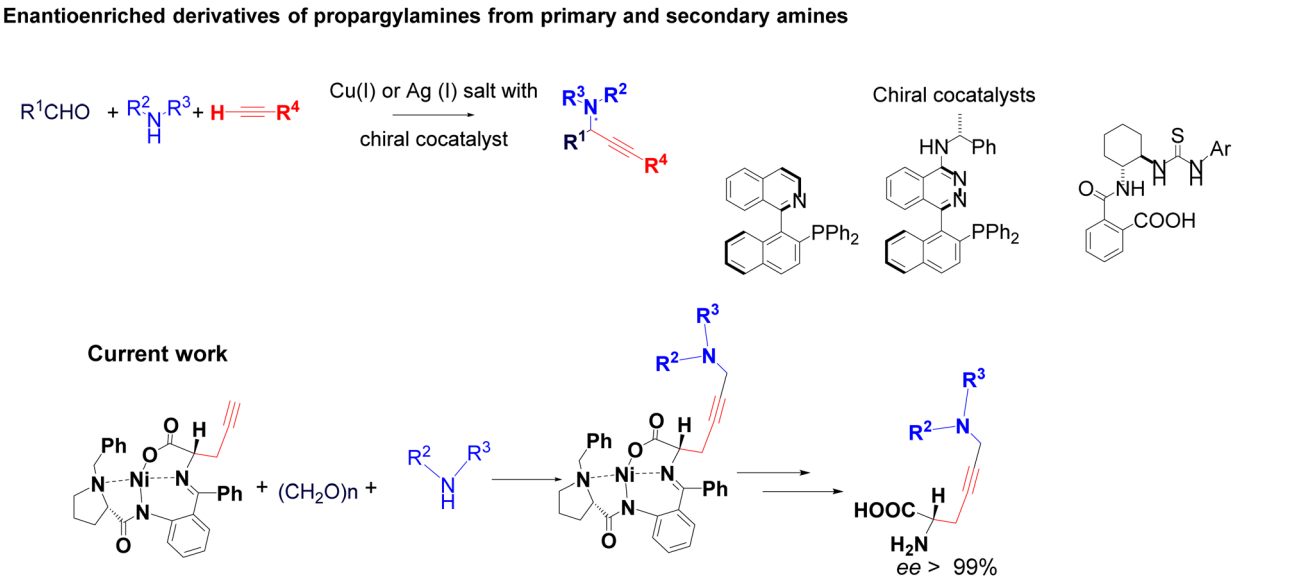
On the other hand, chiral propargylamines play a crucial role as an essential intermediate in the synthesis of biologically active compounds.[14–17] Propargylamine derivatives are commonly obtained through the addition of terminal alkynes to imines or iminium ions[18]. The catalytic enantioselective synthesis of propargylamines from primary amines is a well-established process, often employing copper(I) catalysis with ligands[19]. Copper and silver salts, in combination with various organocatalysts, have also been utilized[20](Scheme 1).

Due to this increased complexity, relatively few studies have tackled the challenge of catalytic enantioselective A^3 reactions with secondary amines[21–23].

[a]*Institute of Pharmacy, Yerevan State University, 1 Alex Manoogian Str., 0025 Yerevan, Armenia. E-mail: anna_mkrtchyan@ysu.am; Fax: +374 60 710 410; Tel: +374 60 710 427*

[b]*SPC "Armbiotechnology" of NAS RA, 14 Gyurjyan Str., 0056 Yerevan, Armenia*

[c]*University of Rostock, Institute of Chemistry, Organic Chemistry, Albert-Einstein-Str. 3a, 18059 Rostock, Germany*



Scheme 1 Synthesis of enantioenriched derivatives of propargylamines.

On the other hand, the combination of three reactants *via* the Mannich reaction is a flexible procedure that has been widely utilized across various applications in organic synthesis.[24–30] The A^3 coupling reactions have undergone thorough examination under both microwave[31–33] and ultrasonic irradiation.[34] These reactions have demonstrated smooth progression in aqueous environments or even in the absence of solvents.[35] They have been applied in the synthesis of natural products, polymers, and biologically active compounds.[36–39]

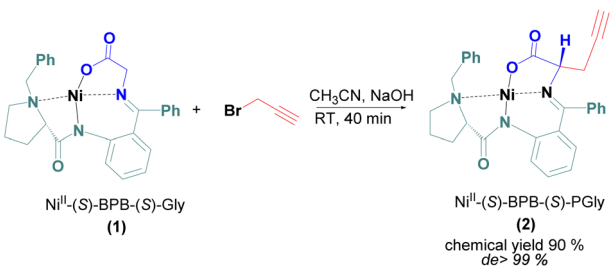
A limited number of studies have reported the application of the Mannich reaction for the synthesis of enantiomerically enriched amino acids.[36–38] However, in most of these cases, the reaction has primarily furnished amino acid derivatives rather than the free amino acids themselves, thereby necessitating an additional synthetic or deprotection step to access the target compounds. Moreover, to the best of our knowledge, no studies have thus far described the direct synthesis of enantiomerically enriched α-amino acids bearing a propargylamine moiety *via* the Mannich reaction. Hence, employing the Mannich reaction for the acquisition of enantiomerically enriched α-amino acids presents a novel and promising avenue.

Additionally, one of the main directions for the synthesis of enantiomerically enriched α-amino acids is stoichiometric asymmetric synthesis using a square-planar nickel Ni(II) of chiral glycine/alanine/dehydroalanine Schiff base comple.[39,40] Numerous research groups have focused on utilizing square-planar Ni(II) complexes of Schiff bases, derived from α-amino acids and chiral carbonyl compounds of (*S*)- and (*R*)-prolines, in stoichiometric asymmetric synthesis. These complexes have been effectively applied in critical reactions such as C_α-alkylation, Michael addition, and aldol reactions, enabling the synthesis of various unnatural amino acids.[41–46] Recently, modified derivatives of Ni(II) square-planar complexes have demonstrated significant success in catalytic transformations, such as Sonogashira,[47] Heck,[48] Suzuki[49] and the copper-catalyzed azide–alkyne cycloaddition reactions.[50] In these processes, the complexes serve as matrices that preserve chirality, ensuring that the chiral center remains unaltered and maintaining stereochemical integrity throughout the catalytic cycle.

Here, we report a hybrid approach that combines both methodologies: utilizing a chiral nickel complex to facilitate enantiomeric yield while exploring the A^3 reaction. Consequently, this approach holds the potential for generating novel enantiomerically enriched non-protein amino acids *via* the A^3 reaction.

Results and discussion

In this study, the Mannich reaction was investigated using a Ni(II) complex of the Schiff base derived from the chiral auxiliary (*S*)-2-*N*-(*N*′-benzylprolyl)aminobenzophenone (BPB) and propargylglycine as the initial complex (**2**)[51] (Scheme 2). A key advantage of this approach lies in its ability to achieve a 99% enantiomeric yield at the C_α – position of the target unnatural α-amino acid, which incorporates a chiral propargylamine moiety. This methodology facilitates the efficient synthesis of the desired product, ensuring a high degree of



Scheme 2 Synthesis of the initial complex **2**.

Table 1 Optimization of reaction conditions A^3 reaction Mannich coupling reaction *via* complex **2**[a]

#	Solvent	**2**: Amine (eq.)	Co-cat.[b]	Time, h	**3a** Conversion, %	**3′** Conversion, %
1	DMSO	1/10	CuI	20	5	0
2	CH_3CN	1/10	CuI	20	10	10
3	CH_3CN	1/20	CuI	20	5	15
4	1,4-Dioxane	1/1.5	$FeCl_3$	16	0	0
5	1,4-Dioxane	1/1.5	CuI	16	20	40
6	1,4-Dioxane	1/1.5	CuI/$FeCl_3$	10	40	30
7	1,4-Dioxane	1/1.5	CuOAc	16	50	50
8	Toluene	1/1.5	CuI/$FeCl_3$	16	65[c]	0
9	Toluene	1/1.5	CuI	16	50	0
10	Toluene	1/1.5	CuI/$FeCl_3$	1.5	79[c]	0

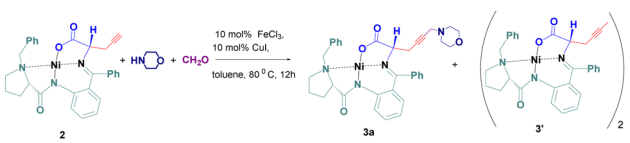
[a] The reaction was carried out under Ar. [b] The co-catalyst is 10 mol/%. [c] The chemical yield was determined by column chromatography.

enantiomeric enrichment (99% ee) at the C_α – position of the α-amino acid.

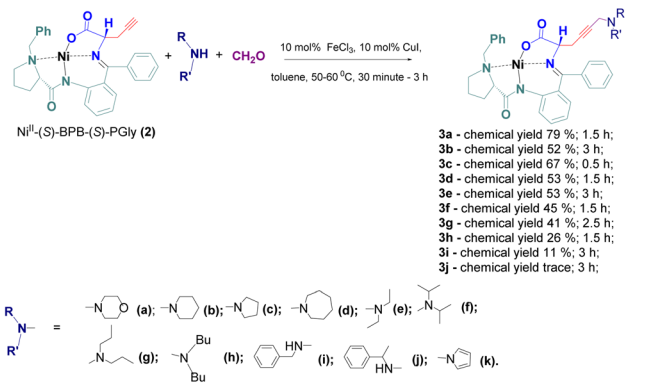
The Mannich reaction was explored through a three-component alkynylation reaction, utilizing paraformaldehyde, complex **2**, and primary or secondary amines. For the optimization conditions as a model reaction was taken a three-component alkynylation process involving paraformaldehyde, morpholine, and complex **2** (Table 1). The progress of the reaction was monitored by TLC (SiO_2, $CHCl_3$/acetone 3 : 1), following the disappearance of the starting complex **2**. During the optimization of the reaction, not only the target product **3a**, but also the dimeric byproduct **3′** was observed (Scheme 3).[52] As side-product analysis confirmed the formation of the dimerized complex **3′**, and given that previous studies have demonstrated the critical role of oxygen as an oxidant in promoting this process, the reaction was therefore carried out under an argon atmosphere to suppress dimerization.[53] Solvent choice exerted a pronounced effect on conversion and selectivity: polar aprotic solvents (DMSO, CH_3CN) led to low to moderate formation of the target product **3a** and, in some cases, increased formation of the dimeric side product **3′**, whereas non-polar or weakly polar solvents (1,4-dioxane, toluene) cauesd the formation of **3a**. The nature of the copper source and the presence of a co-catalyst also significantly influenced the outcome. For example, CuOAc promoted the formation of both **3a** and **3′** (entry 7), while the addition of the Lewis acid $FeCl_3$ enhanced the yield of **3a**. Notably, the combination of CuI and $FeCl_3$ in a non-polar solvent markedly increased the yield of **3a** while suppressing dimerization entirely (entry 8). Shortening the reaction time under these conditions further improved the outcome, as prolonged reaction times promoted side reactions and decomposition of the initial complex **2** (entry 10). Finally, the sequence of reagent addition proved critical: when the catalyst/co-catalyst were present before substrate/amine addition, conversion to **3a** was faster, but extended reaction times increased the proportion of side reactions. In contrast, controlled addition of one reaction partner improved selectivity. Taken together, these observations—solvent polarity, catalyst identity, reaction time, and addition order—defined the optimal conditions outlined in entry 10.

Based on previous studies of Mannich mechanism,[54–56] we can suppose that $FeCl_3$ is proposed to act as a Lewis acid, primarily activating the aldehyde. The amine then adds to the activated aldehyde to form a hemiaminal intermediate, which undergoes $FeCl_3$-facilitated dehydration to generate the iminium ion, the key electrophilic species for subsequent C–C bond formation. Simultaneously, CuI coordinates to the terminal alkyne, forming a π-complex that lowers the pK_a of the terminal proton and enables its deprotonation by the amine or a trace base to generate the nucleophilic Cu–acetylide. This species attacks the electrophilic carbon of the iminium ion, forming the propargylamine framework. Finally, proton transfer restores the neutral amine product, while both $FeCl_3$ and CuI are regenerated to continue the catalytic cycle.

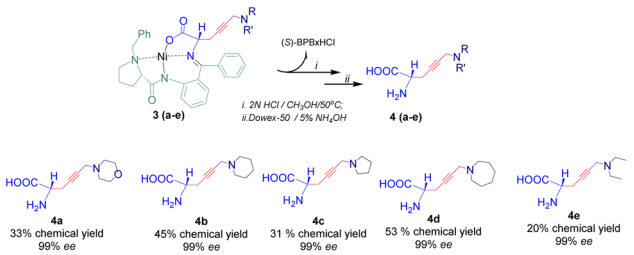
Using optimal conditions various amines were tested (Scheme 4).



Scheme 3 Optimization of A^3 reaction Mannich coupling reaction *via* complex **2**.



Scheme 4 Scope of the A^3 reaction Mannich coupling reaction *via* complex **2**.



Scheme 5 Isolation of the target enantiomerically enriched unnatural α-amino acids.

An interesting pattern was observed regarding the influence of amine type on the chemical yield. 53–79% chemical yields were obtained with cyclic aliphatic amines (Scheme 4, **3a–3d**). However, the yield decreased significantly when transitioning from cyclic to liner aliphatic amines (Scheme 4, **3e–3h**). Furthermore, the nature of the amine played a crucial role; for instance, the yield sharply declined with primary amines. Notably, no reaction occurred in the presence of imidazole. We suppose that imidazole exerts significant electron-donating effects due to its aromatic nitrogen atoms, which could alter the reactivity of the imine intermediate, reducing its reactivity towards nucleophilic attack.

The next step involves the disassembly of some obtained complexes **3(a–e)** and the isolation of α-amino acids. The results of these procedures are summarized in Scheme 5.

In all cases, the asymmetric yield exceeds 99%, as complex **2**, utilized in the reaction, possesses an initial purity of 99%. Although the reaction is carried out in a mildly basic medium, the α-amino acid moiety could, in principle, undergo α-proton elimination; however, experimental evidence confirms stereochemical integrity is retained. However, this possibility can be ruled out, as previous studies have demonstrated that a positive optical rotation in the product complexes is associated with an *S*-absolute configuration at the C_α position of the α-amino acid moiety.[57] Furthermore, X-ray diffraction analysis of intermediate complexes confirms the retention of the *S*-absolute configuration in the products (Fig. 1). To ensure that the absolute configuration remains unaltered throughout the reaction, HPLC analysis of α-amino acid **4a** was performed. The results indicate that the elimination of the α-proton does not occur, confirming the preservation of the absolute configuration and reaffirming that the ee of the obtained amino acids remains above 99%.

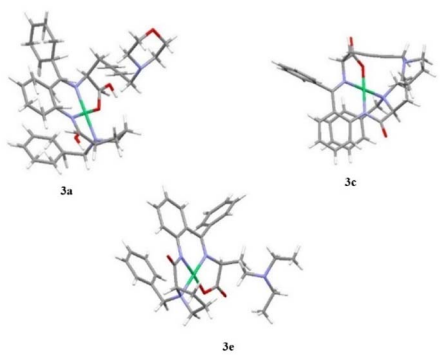


Fig. 1 X-ray analysis of the **3a**, **3c** and **3e**.

Molecular docking analysis

Based on literature data and the recognized potential of unnatural amino acids,[58–60] we decided to evaluate the biological activity of the synthesized unnatural α-amino acids by performing molecular docking studies with collagenase as the target enzyme. This investigation was pursued on two levels: *in silico* docking simulations and *in vitro* enzymatic inhibition assays. According to the molecular docking analysis, all studied structures show an ability to interact with collagenase (Fig. 2). Gibbs free energy values are presented in the Table 2. Of the investigated compounds two bind collagenase in catalytic subdomain very close to the active center (**4d** and **4e**), other two bind to activator domain which is responsible for full activity on collagen[61] (**4a** and **4b**) and remaining one (**4c**) show the ability to interact to helper subdomain. Detailed analysis showed that compounds **4a** and **4c** bind to collagenase by different types of low interactions such as van der Waals forces, hydrophobic interactions *etc.* only. Compound **4b** formed **2** hydrogen bounds by its amino group one with carbonyl oxygen of side carboxyl group of Asp 296 (2.040 Å) and second one with carbonyl oxygen of Gln 215 (1.957 Å). **4d** also formed **2** hydrogen bounds, but one by its amino group with hydroxyl oxygen of Thr 551 (1.776 Å) and another by carboxyl group to amino group of Leu 550 (2.209 Å). Compound **4e** interact to collagenase by forming **3** hydrogen bounds: by carbonyl oxygen of carboxyl group to amino group of Leu 550 (2.201 Å), by hydroxyl oxygen of carboxyl group to a-amino group of Arg 549 (2.139 Å) and by amino group to carbamoyl oxigen of Asn 548 (2.136 Å).

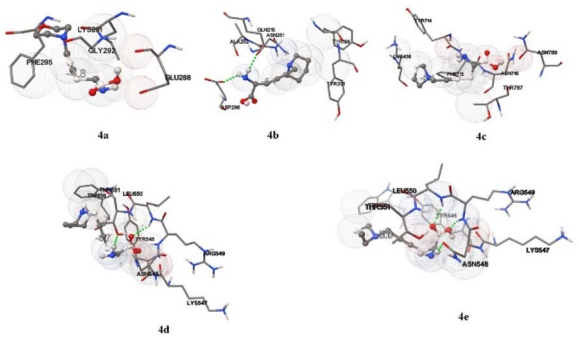


Fig. 2 Molecular Docking of non-protein α-amino acids **4a–4e**.

Table 2 Results of the molecular docking analysis and biological analysis

	Compound	Gibbs free energy kcal mol^{-1}	IC_{50}
1	**4a**	−4.8	—
2	**4b**	−5.4	2.27
3	**4c**	−5.4	1.52
4	**4d**	−5.7	1.225
5	**4e**	−5	0.75

Inhibition assays on the collagenase activity

To validate the *in silico* findings, all synthesized compounds were subjected to enzymatic inhibition assays targeting collagenase. The activity of collagenase has been determined in the presence of investigated amino acids. Investigated amino acids were added in the concentration range 0.2–2 mM. The obtained results are presented in the Table 2. According to the obtained results, collagenase has been inhibited by all investigated compounds except **4a**.

Although compound **4d** exhibits a more favorable Gibbs free energy *in silico*, compound **4e** shows superior *in vitro* inhibition (lowest IC_{50}). This discrepancy highlights the limitations of docking scores as sole predictors of biological activity and underscores the importance of specific hydrogen bonding networks, binding site geometry, and dynamic enzyme–ligand interactions, which may not be fully captured in docking simulations. The enhanced activity of **4e** likely results from its favorable binding orientation and extensive hydrogen bonding within the catalytic subdomain, effectively impairing collagenase function.

Experimental

General information

All amines, aldehydes were obtained from commercial sources and used without further purification. The initial **2** complexes were prepared following literature protocols.[51] TLC analyses were performed on glass plates coated with silica gel 60 F254. Column chromatography was performed on silica gel (60 × 120 mesh) on a glass column.

Instrumentation

Melting points (mp) were determined by ⟪Electrothermal⟫. ^{1}H and ^{13}C NMR spectra (⟪Mercury-300 Varian⟫ 300 MHz respectively) were recorded using TMS as an internal standard (0 ppm). Elemental analyses were done by elemental analyzer EURO EA 3000. The enantiomeric purity of the amino acids was determined by HPLC (⟪Waters Alliance 2695 HPLC System⟫) on the chiral phase Diaspher-110- Chirasel-E-PA 6 : 0 mkm 4.0 × 250 mm, and a mixture of 20% MeOH and 80% 0 : 1 M aqueous solution $NaH_2PO_4 \cdot 2H_2O$ was used as the eluent. The optical rotation was measured on a Perkin Elmer-341 polarimeter, The X-ray was done by Enraf-Nonius CAD4, LCMS analysis was done by Shimadzu LCMS 2020 with prominence-I LC-2030C 3D. The CD analyses was done my Chirascan™ V100.

Genral procedure of Mannich reaction with paraformaldehyde

A 2 g (0.0037 mol) sample of Ni^{II}-(*S*)-BPB-(*S*)-PGly complex, 0.336 g (0.011 mol) of paraformaldehyde, and 0.060 g (0.00037 mol) of $FeCl_3$ were added to a round-bottom flask connected to a reflux condenser and supplied with an argon gas flow. The mixture was dissolved in 20 mL toluene for 15–20 minutes. Then, 0.07 g (0.00037 mol) of CuI and (0.0074 mol) of amine mixed with a small amount of toluene were added. The reaction mixture was stirred under heating conditions at a temperature of 50–60 °C. The course of the reaction was monitored by TLC (SiO_2, $CHCl_3/CH_3COCH_3$ = 3/1) until the traces of the initial complex were eliminated. Based on TLC data, the reaction took 0.5–3 h.

After completion of the reaction, the mixture was poured into distilled water (50 mL) under stirring for 15 min and extracted with methylene chloride (3 × 30 mL). The combined organic layers were washed with distilled water (2 × 20 mL), dried over anhydrous $MgSO_4$ for 30 min, and filtered. The solvent was removed under reduced pressure, and the residue was crystallized from acetone (20 mL) to afford the pure product.

General procedure for isolation of amino acids

Complexes **3a–d** were dissolved in MeOH (50 mL) and slowly added to 2 M HCl (50 mL). The reaction mixture was heated at 50 °C until the characteristic red color of the metal complex disappeared. The solution was then concentrated under reduced pressure, diluted with water (50 mL), and the precipitated (*S*)-BPB HCl was collected by filtration. The optically active α-amino acids **4a–d** were subsequently isolated from the aqueous layer by ion-exchange chromatography on Dowex-50 (H^+ form) resin, using 5% aqueous NH_4OH as the eluent. The eluates were concentrated under reduced pressure, and the amino acids were purified by recrystallization from a water/EtOH mixture (1 : 2 v/v).

The obtained amino acids **4(a–d)** were practically insoluble in any solvent, which caused difficulties for measuring their specific rotation.

Molecular docking

The structures of the compounds were build using ChemBioOffice 2010 (ChemBio3D Ultra 12.0). Minimization of the ligand free energy was performed using the MM2 force field and the truncated Newton–Raphson method. The crystallographic structure of collagenase G (PDB ID: 2Y50) was used in analysis. Water molecules were removed, and polar hydrogens were added according to software producer's suggested protocol of macromolecule preparation. Docking of ligands to the enzyme was performed using AutoGrid 4 and AutoDock Vina software[62]. Ligands were ranked based on an energy-dependent score function, and protein–ligand interactions were modeled on a grid to speed up calculations. Interaction modes were identified as well as bond types and lengths were determined.

Determination of collagenase activity

Collagenase activity was determined by a method based on the measurement of free amino groups that are released during substrate hydrolysis.[63] The reaction mixture consisted of 0.05 M HEPES buffer (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) with pH 7.2, 10 mg mL^{-1} gelatin and 0.025 mg mL^{-1} collagenase, the reaction was carried out at 37 °C.

To determine the concentration of amino groups, the *ortho*-phthalaldehyde (OPA) reagent was used consisting of 0.2 M borate buffer with pH 9.7, 0.1667 mg mL^{-1} OPA and 1.18 mM mercaptoethanol. Aliquots of 50 μL were taken every 30

minutes, and the reaction was stopped by adding 10 μL of 30% trichloroacetic acid. Then 1.5 mL of OPA reagent and 1.5 mL of water were added to the resulting sample, and the A340 value was recorded after 5-minutes incubation at a temperature of 27 °C.

Conclusions

This study presents a novel hybrid synthetic strategy that integrates the use of chiral square-planar Ni(II) Schiff base complexes with the A^3 Mannich reaction, enabling the efficient synthesis of enantiomerically enriched unnatural α-amino acids containing different type of moiety. The optimized conditions using a CuI/$FeCl_3$ co-catalyst system in toluene under inert atmosphere resulted in high chemical yields and excellent stereoselectivity (≥99% ee) at the C_α-position. The synthetic approach was found to be particularly efficient with cyclic secondary amines, while linear or primary amines significantly reduced the yields.

Subsequent structural elucidation and chiral purity validation, including HPLC and X-ray crystallography, confirmed the preservation of the *S*-absolute configuration during synthesis. Furthermore, the biological potential of the synthesized amino acids was explored through molecular docking and *in vitro* inhibition assays against collagenase.

Although docking analysis revealed favorable Gibbs free energy values and binding interactions—particularly extensive hydrogen bonding in compound **4e**—the biological assays highlighted the complexity of enzyme–ligand dynamics. Notably, compound **4e**, despite not having the lowest docking score, exhibited the highest inhibitory activity ($IC_{50} = 0.75$ mM), demonstrating that docking energy alone is not always a reliable predictor of *in vitro* efficacy. These findings emphasize the necessity of integrating computational and experimental evaluations for accurate assessment of bioactivity.

Overall, the developed approach not only provides a robust route to stereochemically pure non-proteinogenic α-amino acids but also yields biologically active compounds with potential applications in enzyme inhibition and medicinal chemistry.

Author contributions

Liana Hayriyan contributed to the methodology, investigation, data curation. Anna Grigoryan was involved in the investigation, validation, data curation. Hasmik Gevorgyan contributed to formal analysis, visualization. Avetis Tsaturyan participated in the investigation, provided resources, and supported project administration. Armen Sargsyan was responsible for software, formal analysis, and validation. Peter Langer contributed to supervision, writing – review and editing. Ashot Saghyan supervision, project administration, and funding acquisition. Anna Mkrtchyan was involved in conceptualization, data interpretation, writing – original draft, writing – review and editing and supervision.

Conflicts of interest

There are no conflicts to declare.

Data availability

Additional data related to molecular docking studies are available from the corresponding author upon reasonable request.

CCDC 2355349, 2355346 and 2355404 contain the supplementary crystallographic data for this paper.[64a–c]

All experimental data supporting the findings of this study, including synthetic procedures, characterization data (NMR, HPLC, X-ray crystallography), and biological assay results, are provided in the SI. Supplementary information: detailed experimental procedures, instrumentation details, NMR and MS spectra, HPLC analysis, X-ray crystallographic data, and molecular docking studies. See DOI: **https://doi.org/10.1039/d5ra04554a**.

Acknowledgements

This work was supported by the Higher Education and Science Committee of MESCS RA in the frames of the research projects 24LCG-1D018 and ISTC AM-2705.

References

1 C. Najera and J. M. Sansano, Catalytic Asymmetric Synthesis of Alpha-Amino Acids, *Chem. Rev.*, 2007, **107**(11), 4584–4671, DOI: **10.1021/cr050580o**.

2 Z. Li and C.-J. Li, Asymmetric Synthesis Based on Catalytic Activation of C–H Bonds and C–C Bonds, in *New Frontiers in Asymmetric Catalysis*, John Wiley & Sons, Ltd, 2007, pp. 129–152, DOI: **10.1002/9780470098004.ch5**.

3 E. N. Jacobsen, A. Pfaltz and H. Yamamoto, *Comprehensive Asymmetric Catalysis*, Springer, 2003.

4 K. K. Sharma, K. Sharma, K. Rao, A. Sharma, G. K. Rathod, S. Aaghaz, N. Sehra, R. Parmar, B. VanVeller and R. Jain, Unnatural Amino Acids: Strategies, Designs, and Applications in Medicinal Chemistry and Drug Discovery, *J. Med. Chem.*, 2024, **67**(22), 19932–19965, DOI: **10.1021/acs.jmedchem.4c00110**.

5 I. Maluch, J. Czarna and M. Drag, Applications of Unnatural Amino Acids in Protease Probes, *Chem. Asian J.*, 2019, **14**(23), 4103–4113, DOI: **10.1002/asia.201901152**.

6 T. Narancic, S. A. Almahboub and K. E. O'Connor, Unnatural Amino Acids: Production and Biotechnological Potential, *World J. Microbiol. Biotechnol.*, 2019, **35**(4), 67, DOI: **10.1007/s11274-019-2642-9**.

7 *Antimicrobial Peptides: An Evolving Treasure to Combat Emerging Infections and Drug-resistance*, SpringerLink, **https://link.springer.com/collections/ihefgacjfj**, accessed 2025-09-02.

8 *Unusual Amino Acids in Medicinal Chemistry | Journal of Medicinal Chemistry*, **https://pubs.acs.org/doi/abs/10.1021/acs.jmedchem.6b00319**, accessed 2025-09-02.

9 M. A. Fik-Jaskółka, A. F. Mkrtchyan, A. S. Saghyan, R. Palumbo, A. Belter, L. A. Hayriyan, H. Simonyan, V. Roviello and G. N. Roviello, Spectroscopic and SEM Evidences for G4-DNA Binding by a Synthetic Alkyne-Containing Amino Acid with Anticancer Activity, *Spectrochim. Acta, Part A*, 2020, **229**, 117884, DOI: **10.1016/j.saa.2019.117884**.

10 V. Roviello, D. Musumeci, A. Mokhir and G. N. Roviello, Evidence of Protein Binding by a Nucleopeptide Based on a Thyminedecorated L-Diaminopropanoic Acid through CD and In Silico Studies, **https://www.eurekaselect.com**.

11 *Synthesis, biological evaluation and supramolecular assembly of novel analogues of peptidyl nucleosides - Molecular BioSystems*, RSC Publishing, **https://pubs.rsc.org/en/content/articlelanding/2011/mb/c1mb05007a**, accessed 2025-09-03.

12 *Synthesis, characterization and hybridization studies of an alternate nucleo-ε/γ-peptide: complexes formation with natural nucleic acids | Amino Acids*, **https://link.springer.com/article/10.1007/s00726-008-0214-7**, accessed 2025-09-03.

13 H. Simonyan, R. Palumbo, C. Vicidomini, P. L. Scognamiglio, S. Petrosyan, L. Sahakyan, G. Melikyan, A. Saghyan and G. N. Roviello, Binding of G-Quadruplex DNA and Serum Albumins by Synthetic Non-Proteinogenic Amino Acids: Implications for c-Myc-Related Anticancer Activity and Drug Delivery, *Mol. Ther. Nucleic Acids*, 2025, **36**(2), 102478, DOI: **10.1016/j.omtn.2025.102478**.

14 Y. Hayashi, T. Yamazaki, G. Kawauchi and I. Sato, Prolinate Salt as a Catalyst in the Syn-Selective, Asymmetric Mannich Reaction of Alkynyl Imine, *Org. Lett.*, 2018, **20**(8), 2391–2394, DOI: **10.1021/acs.orglett.8b00728**.

15 Y. Zhao, J. Li, R. Ma, F. He, H. Shi, X. Duan, H. Li, X. Xie and X. She, Ten-Step Total Synthesis of (−)-Andranginine, *Org. Lett.*, 2022, **24**(37), 6880–6883, DOI: **10.1021/acs.orglett.2c02927**.

16 J. Ying and L. Pu, Regiospecific Hydration of N-(Diphenylphosphinoyl)Propargyl Amines: Synthesis of β-Amino Ketones by Au(III) Catalysis, *J. Org. Chem.*, 2016, **81**(18), 8135–8141, DOI: **10.1021/acs.joc.6b01111**.

17 E. Ramos, R. Lajarín-Cuesta, R. L. Arribas, E. M. García-Frutos, L. González-Lafuente, J. Egea, C. de los Ríos and A. Romero, Silico Prediction of the Toxic Potential of Neuroprotective Bifunctional Molecules Based on Chiral N-Propargyl-1,2-Amino Alcohol Derivatives, *Chem. Res. Toxicol.*, 2021, **34**(5), 1245–1249, DOI: **10.1021/acs.chemrestox.0c00519**.

18 *Enantioselective A3 Reactions of Secondary Amines with a Cu(I)/Acid–Thiourea Catalyst Combination | Journal of the American Chemical Society*, **https://pubs.acs.org/doi/10.1021/jacs.5b02071**, accessed 2025-09-03.

19 *Cu-Catalyzed Direct Asymmetric Mannich Reaction of 2-Alkylazaarenes and Isatin-Derived Ketimines | Organic Letters*, **https://pubs.acs.org/doi/10.1021/acs.orglett.4c00227**, accessed 2025-09-03.

20 G. Fang and X. Bi, Silver-Catalysed Reactions of Alkynes: Recent Advances, *Chem. Soc. Rev.*, 2015, **44**(22), 8124–8173, DOI: **10.1039/C5CS00027K**.

21 *Development of Catalytic Enantioselective Mannich Reactions Using Esters | Organic Letters*, **https://pubs.acs.org/doi/10.1021/acs.orglett.3c04326**, accessed 2025-09-03.

22 *Organocatalyzed Asymmetric Mannich Reaction: An Update - Bagheri - 2021 - ChemistrySelect*, Wiley Online Library, **https://chemistry-europe.onlinelibrary.wiley.com/doi/10.1002/slct.202003034**, accessed 2025-09-03.

23 *Recent Advances in Squaramide-Catalyzed Asymmetric Mannich Reactions - Hou - 2020 - Advanced Synthesis & Catalysis*, Wiley Online Library, **https://advanced.onlinelibrary.wiley.com/doi/10.1002/adsc.202000842**, accessed 2025-09-03.

24 *A Review on Recent Advances in the Application of Nanocatalysts in A^3 Coupling Reactions - Nasrollahzadeh - 2018 - The Chemical Record*, Wiley Online Library, **https://onlinelibrary.wiley.com/doi/10.1002/tcr.201700100** accessed 2025-09-03.

25 A. Grirrane, E. Álvarez, H. García and A. Corma, Cationic Copper(I) Complexes as Highly Efficient Catalysts for Single and Double A3-Coupling Mannich Reactions of Terminal Alkynes: Mechanistic Insights and Comparative Studies with Analogous Gold(I) Complexes, *Chem.–Eur. J.*, 2014, **20**(44), 14317–14328, DOI: **10.1002/chem.201403927**.

26 *A3 Coupling Reaction in the Synthesis of Heterocyclic Compounds - Volkova - 2021 - Advanced Synthesis & Catalysis*, Wiley Online Library, **https://advanced.onlinelibrary.wiley.com/doi/10.1002/adsc.202000866**, accessed 2025-09-03.

27 *Metal-Catalysed A3 Coupling Methodologies: Classification and Visualisation*, **https://www.mdpi.com/2073-4344/12/6/660**, accessed 2025-09-03.

28 *CuI-Catalyzed C1-Alkynylation of Tetrahydroisoquinolines (THIQs) by A^3 Reaction with Tunable Iminium Ions | Organic Letters*, **https://pubs.acs.org/doi/10.1021/ol402517e**, accessed 2025-09-03.

29 *Recent Advances in the A^3 Coupling Reactions and their Applications - Jesin - 2019 - European Journal of Organic Chemistry*, Wiley Online Library, **https://chemistry-europe.onlinelibrary.wiley.com/doi/10.1002/ejoc.201900001**, accessed 2025-09-03.

30 G. Roman, Mannich Bases in Medicinal Chemistry and Drug Design, *Eur. J. Med. Chem.*, 2015, **89**, 743–816, DOI: **10.1016/j.ejmech.2014.10.076**.

31 *Amine-Functionalized Mesoporous Silica as a Support for on-Demand Release of Copper in the A^3-Coupling Reaction: Ultralow Concentration Catalysis and Confinement Effect | ACS Sustainable Chemistry & Engineering*, **https://pubs.acs.org/doi/10.1021/acssuschemeng.9b00576**, accessed 2025-09-03.

32 *Propargyl Amine Synthesis Catalysed by Gold and Copper Thin Films by Using Microwave-Assisted Continuous-Flow Organic Synthesis (MACOS) - Shore - 2010 - Chemistry – A European Journal*, Wiley Online Library, **https://chemistry-**

europe.onlinelibrary.wiley.com/doi/10.1002/chem.200902396, accessed 2025-09-03.

33 V. S. Kashid and M. S. Balakrishna, Microwave-Assisted Copper(I) Catalyzed A3-Coupling Reaction: Reactivity, Substrate Scope and the Structural Characterization of Two Coupling Products, *Catal. Commun.*, 2018, **103**, 78–82, DOI: 10.1016/j.catcom.2017.09.020.

34 *Ultrasonic and bio-assisted synthesis of Ag@HNTs-T as a novel heterogeneous catalyst for the green synthesis of propargylamines: A combination of experimental and computational study - Malmir - 2018 - Applied Organometallic Chemistry*, Wiley Online Library, https://onlinelibrary.wiley.com/doi/10.1002/aoc.4291, accessed 2025-09-03.

35 *Thieme E-Journals - Synthesis/Abstract*, https://www.thieme-connect.de/products/ejournals/abstract/10.1055/s-0037-1612253, accessed 2025-09-03.

36 P. Pecchini, M. Fochi, F. Bartoccini, G. Piersanti and L. Bernardi, Enantioselective Organocatalytic Strategies to Access Noncanonical α-Amino, *Acids*, 2024, 20365, DOI: 10.1039/D4SC01081G.

37 A. M. Faisca Phillips, M. F. C. Guedes da Silva and A. J. L. Pombeiro, The Stereoselective Nitro-Mannich Reaction in the Synthesis of Active Pharmaceutical Ingredients and Other Biologically Active Compounds, *Front. Chem.*, 2020, **8**, 30, DOI: 10.3389/fchem.2020.00030.

38 Y. Zhang, J. Vanderghinste, J. Wang and S. Das, Challenges and Recent Advancements in the Synthesis of α,α-Disubstituted α-Amino Acids, *Nat. Commun.*, 2024, **15**(1), 1474, DOI: 10.1038/s41467-024-45790-2.

39 A. S. Dadayan, A. F. Mkrtchyan, A. S. Poghosyan, S. A. Dadayan, L. A. Stepanyan, M. H. Israyelyan, A. S. Tovmasyan, A. H. Tsaturyan, N. A. Hovhannisyan, V. O. Topuzyan, T. H. Sargsyan and A. S. Saghyan, Unnatural Phosphorus-Containing α-Amino Acids and Their N-FMOC Derivatives: Synthesis and In Vitro Investigation of Anticholinesterase Activity, *ChemistrySelect*, 2024, **9**(3), e202303249, DOI: 10.1002/slct.202303249.

40 *Asymmetric synthesis of pyroglutamic acids via Ni(II) complex methodology | Chemistry of Heterocyclic Compounds*, https://link.springer.com/article/10.1007/s10593-023-03203-0, accessed 2025-09-05.

41 A. S. Dadayan, A. F. Mkrtchyan, A. S. Poghosyan, S. A. Dadayan, L. A. Stepanyan, M. H. Israyelyan, A. S. Tovmasyan, A. H. Tsaturyan, N. A. Hovhannisyan, V. O. Topuzyan, T. H. Sargsyan and A. S. Saghyan, Unnatural Phosphorus-Containing α-Amino Acids and Their N-FMOC Derivatives: Synthesis and In Vitro Investigation of Anticholinesterase Activity, *ChemistrySelect*, 2024, **9**(3), e202303249, DOI: 10.1002/slct.202303249.

42 N. V. Lyutenko, A. E. Sorochinsky and V. A. Soloshonok, Asymmetric Synthesis of Pyroglutamic Acids via Ni(II) Complex Methodology, *Chem. Heterocycl. Compd.*, 2023, **59**(6), 332–340, DOI: 10.1007/s10593-023-03203-0.

43 Y. Zou, Z. Yin, H. Mei, H. Konno, H. Moriwaki, V. A. Soloshonok and J. Han, Aldol Addition-Cyclization Reaction Cascade on a Platform of Chiral Ni(II) Complex of Glycine Schiff Base, *Ukr. Bioorg. Acta*, 2021, **16**(1), 3–9, DOI: 10.15407/bioorganica2021.01.003.

44 *BSA Binding and Aggregate Formation of a Synthetic Amino Acid with Potential for Promoting Fibroblast Proliferation: An In Silico, CD Spectroscopic, DLS, and Cellular Study*, https://www.mdpi.com/2218-273X/14/5/579, accessed 2025-09-05.

45 Z. T. Gugkaeva, M. P. Stukalova, A. F. Smol'yakov, A. T. Tsaloev, V. I. Maleev and V. A. Larionov, Asymmetric Metal-Templated Approach to Amino Acids with a CF3-Containing 3,2'-Pyrrolidinyl Spirooxindole Core via a Michael/Mannich [3+2]-Cycloaddition Reaction, *Adv. Synth. Catal.*, 2024, **366**(5), 1205–1211, DOI: 10.1002/adsc.202301214.

46 N. V. Stoletova, A. F. Smol'yakov, A. A. Tyutyunov, V. I. Maleev and V. A. Larionov, A Radical Hydrohaloalkylation of the Ligand Sphere of a Chiral Dehydroalanine Ni(II) Complex: An Asymmetric Route to Halogenated α-Amino Acid Derivatives, *Tetrahedron Chem*, 2025, **13**, 100118, DOI: 10.1016/j.tchem.2024.100118.

47 *Synthesis of Enantiomerically Enriched Alkynylaryl-Substituted α-Amino Acids through Sonogashira Reactions - Saghyan - 2019 - ChemistrySelect*, Wiley Online Library, https://chemistry-europe.onlinelibrary.wiley.com/doi/10.1002/slct.201903072, accessed 2025-09-05.

48 *Thieme E-Journals - Synlett/Abstract*, https://www.thieme-connect.de/products/ejournals/abstract/10.1055/a-1941-2068, accessed 2025-09-05.

49 *Synthesis of Enantiomerically Enriched Non-Proteinogenic α-Amino Acids Using the Suzuki Reaction - Saghyan - 2019 - ChemistrySelect*, Wiley Online Library, https://chemistry-europe.onlinelibrary.wiley.com/doi/10.1002/slct.201900698, accessed 2025-09-05.

50 *Synthesis of enantiomerically enriched β-substituted analogs of (S)-α-alanine containing 1-phenyl-1H-1,2,3-triazole groups | Amino Acids*, https://link.springer.com/article/10.1007/s00726-024-03430-5, accessed 2025-09-05.

51 S. Collet, P. Bauchat, R. Danion-Bougot and D. Danion, Stereoselective, Nonracemic Synthesis of ω-Borono-α-Amino Acids, *Tetrahedron: Asymmetry*, 1998, **9**(12), 2121–2131, DOI: 10.1016/S0957-4166(98)00186-4.

52 A. F. Mkrtchyan, L. A. Hayriyan, A. J. Karapetyan, A. S. Tovmasyan, A. H. Tsaturyan, V. N. Khrustalev, V. I. Maleev and A. S. Saghyan, Using the Ni-[(Benzylprolyl) Amino]Benzophenone Complex in the Glaser Reaction for the Synthesis of Bis α-Amino Acids, *New J. Chem.*, 2020, **44**(28), 11927–11932, DOI: 10.1039/D0NJ02072A.

53 *Glaser Heterocoupling Reaction for the Synthesis of Enantiomerically Enriched Unnatural α-Amino Acids Incorporating Asymmetric Diyne Moieties: Mechanistic Insights and Optimization | ACS Omega*, https://pubs.acs.org/doi/10.1021/acsomega.4c10524, accessed 2025-09-06.

54 *Enantioselective One-Pot Three-Component Synthesis of Propargylamines | Organic Letters*, https://pubs.acs.org/doi/10.1021/ol060793f, accessed 2025-09-07.

55 A. Grirrane, E. Álvarez, H. García and A. Corma, Cationic Copper(I) Complexes as Highly Efficient Catalysts for Single and Double A3-Coupling Mannich Reactions of Terminal Alkynes: Mechanistic Insights and Comparative Studies with Analogous Gold(I) Complexes, *Chem.–Eur. J.*, 2014, **20**(44), 14317–14328, DOI: **10.1002/chem.201403927**.

56 *The Asymmetric A3(Aldehyde–Alkyne–Amine) Coupling: Highly Enantioselective Access to Propargylamines*, **https://www.mdpi.com/1420-3049/24/7/1216**, accessed 2025-09-07.

57 *Asymmetric Synthesis of Non-Proteinogenic Amino Acids | Wiley*, Wiley.com, **https://www.wiley.com/en-us/Asymmetric+Synthesis+of+Non-Proteinogenic+Amino+Acids-p-9783527804498**, accessed 2025-09-05.

58 A. S. Sargsyan, A. J. Karapetyan, L. A. Hayriyan, A. M. Hovhannisyan, M. A. Melkumyan, G. G. Oganezova, N. S. Avetisyan and N. A. Hovhannisyan, The Influence Of Non-Protein Amino Acids And Peptides On Bacterial Collagenase, *Proc. YSU B: Chem. Biol. Sci.*, 2022, **56**(2), 126–130, DOI: **10.46991/PYSU:B/2022.56.2.126**.

59 Z.-Z. Wang, K. Wang, L.-F. Xu, C. Su, J.-S. Gong, J.-S. Shi, X.-D. Ma, N. Xie and J.-Y. Qian, Unlocking the Potential of Collagenases: Structures, Functions, and Emerging Therapeutic Horizons, *BioDesign Res.*, 2024, **6**, 0050, DOI: **10.34133/bdr.0050**.

60 A. Hartmann, J. Gostner, J. E. Fuchs, E. Chaita, N. Aligiannis, L. Skaltsounis and M. Ganzera, Inhibition of Collagenase by Mycosporine-like Amino Acids from Marine Sources, *Planta Med.*, 2015, **81**(10), 813–820, DOI: **10.1055/s-0035-1546105**.

61 U. Eckhard, E. Schönauer and H. Brandstetter, Structural Basis for Activity Regulation and Substrate Preference of Clostridial Collagenases G, H, and T*, *J. Biol. Chem.*, 2013, **288**(28), 20184–20194, DOI: **10.1074/jbc.M112.448548**.

62 *AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading - Trott - 2010 - Journal of Computational Chemistry*, Wiley Online Library, **https://onlinelibrary.wiley.com/doi/10.1002/jcc.21334**, accessed 2025-09-05.

63 *Modeling, synthesis and in vitro screening of unusual amino acids and peptides as protease inhibitors | Journal of Chemical Technology and Metallurgy*, **https://j.uctm.edu/index.php/JCTM/article/view/93**, accessed 2025-09-05.

64 (*a*) L. Hayriyan, A. Grigoryan, H. Gevorgyan, A. Tsaturyan, A. Sargsyan, P. Langer, A. Saghyan, A. Mkrtchyan, CCDC 2355349: Experimental Crystal Structure Determination, 2025, DOI: **10.5517/ccdc.csd.cc2k1y0l**; (*b*) L. Hayriyan, A. Grigoryan, H. Gevorgyan, A. Tsaturyan, A. Sargsyan, P. Langer, A. Saghyan, A. Mkrtchyan, CCDC 2355346: Experimental Crystal Structure Determination, 2025, DOI: **10.5517/ccdc.csd.cc2k1xxg**; (*c*) L. Hayriyan, A. Grigoryan, H. Gevorgyan, A. Tsaturyan, A. Sargsyan, P. Langer, A. Saghyan, A. Mkrtchyan, CCDC 2355404: Experimental Crystal Structure Determination, 2025, DOI: **10.5517/ccdc.csd.cc2k1zsd**.