

RSC Advances



This is an *Accepted Manuscript*, which has been through the Royal Society of Chemistry peer review process and has been accepted for publication.

Accepted Manuscripts are published online shortly after acceptance, before technical editing, formatting and proof reading. Using this free service, authors can make their results available to the community, in citable form, before we publish the edited article. This *Accepted Manuscript* will be replaced by the edited, formatted and paginated article as soon as this is available.

You can find more information about *Accepted Manuscripts* in the [Information for Authors](#).

Please note that technical editing may introduce minor changes to the text and/or graphics, which may alter content. The journal's standard [Terms & Conditions](#) and the [Ethical guidelines](#) still apply. In no event shall the Royal Society of Chemistry be held responsible for any errors or omissions in this *Accepted Manuscript* or any consequences arising from the use of any information it contains.

Synthesis of Optically Pure (*S*)-2-Amino-5-aryl-pent-4-ynoic acids by Sonogashira Reactions and their Potential Use as Highly Selective Potent Inhibitors of Aldose Reductase

Silvio Parpart,^a Andranik Petrosyan,^{a,b,c} Syed Jawad Ali Shah,^c Raji Akeem Adewale,^c Peter Ehlers,^a Tatevik Grigoryan,^{a,d} Anna F. Mkrтчhyan,^{c,d} Zorayr Z. Mardiyan,^c Ani J. Karapetyan,^c Avetis H. Tsaturyan,^c Ashot S. Saghyan,^{c,d} Jamshed Iqbal,^c Peter Langer*^{a,b}

^a Universität Rostock, Institut für Chemie, A.-Einstein-Str. 3a, 18059 Rostock, Germany

E-Mail: peter.langer@uni-rostock.de

^b Leibniz-Institut für Katalyse e.V. an der Universität Rostock, A.-Einstein-Str. 29a, 18059 Rostock, Germany

^c SPC “Armbiotechnology” SNPO NAS RA, Gyurjyan Str. 14, 0056 Yerevan, Armenia

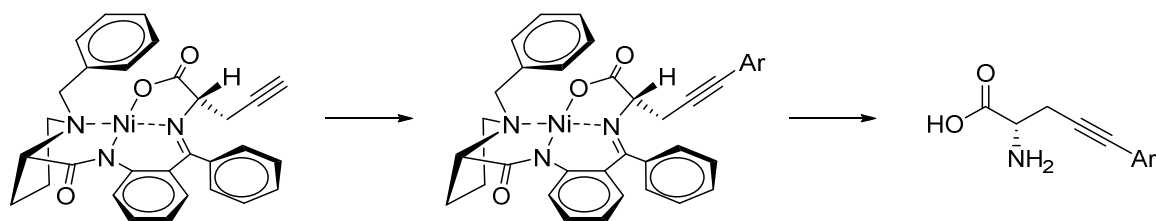
^d Yerevan State University, Faculty of Pharmacology and Chemistry, Alex Manoogian Str. 1, 0025 Yerevan, Armenia

^e Centre for Advanced Drug Research, COMSATS Institute of Information Technology,

22060 Abbottabad, Pakistan

E-Mail: drjamshed@ciit.net.pk

Abstract: A new and convenient synthesis of optically pure (*S*)-2-amino-5-[aryl]pent-4-ynoic acids (alkynylated amino acids) is reported. (*S*)-2-Aminopent-4-ynoic acid-Ni(*S*)-BPB was prepared and then functionalized via Sonogashira cross-coupling reactions to give (*S*)-2-amino-5-[aryl]pent-4-ynoic acid-Ni(*S*)-BPB. The reaction conditions for the cross-coupling reaction were optimized and twelve derivatives were synthesized. Afterwards the Ni-complexes were cleaved to obtain the free (*S*)-2-amino-5-[aryl]pent-4-ynoic acids with excellent optical purity. The prepared (*S*)-2-amino-5-[aryl]pent-4-ynoic acids were tested for biological activity and selectively showed high inhibitory activity against aldose reductase (ALR2) over ALR1. Molecular docking study have also been carried out to identify the putative binding mode of the compounds in these enzymes.



Keywords: cross-coupling, palladium, Sonogashira reaction, amino acids, asymmetric synthesis, aldehyde reductase inhibitors, molecular docking

Introduction

Amino acids and peptides are of great significance in life sciences and can be found in many areas of everyday life. Thus, the application of amino acids and their oligomers or polymers range from biotechnology, medicine, microbiology to food industry. Some examples are given in Figure 1. L-Thyroxine is the treatment of choice for patients with hypothyroidism. L-Glutamic acid is used as flavor-enhancing compound which provide an umami (savory) taste to food. While its activity is similar to normal L-DOPA, the 6-fluorinated L-DOPA can be used as radiolabeled molecular imaging probe useful for *in vivo* imaging for biological research and drug discovery. Consequently, many strategies for the synthesis of amino acids and their derivatives have been developed since the first synthesis of Alanin by Strecker in 1850.¹ In contrast to the classical synthetic routes, modern chemistry focuses on the synthesis of optically pure amino acids. Therefore, a chiral substrate, reagent, catalyst or environment is required to perform the asymmetric induction.²

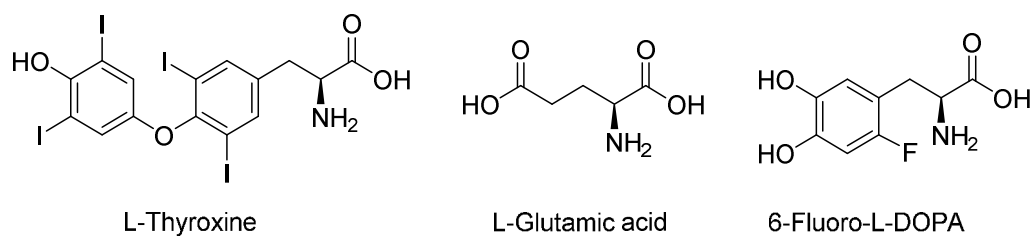
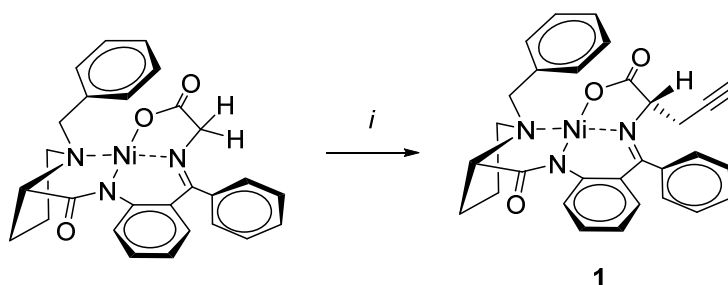


Figure 1: Examples for amino acids and their applications.

In line with this background, amino acid-Ni-BPB complexes (BPB = N-(benzylpropyl)aminobenzophenone) were developed in the last decades as a chiral starting material.³ The chiral auxiliary BPB performs the asymmetric induction, while Ni increases the

stability and reactivity of the amino acid-BPB adduct. The amino acid-Ni-BPB complex is stable to air and moisture and it is synthesized starting from easily available building blocks (benzylchlorid, proline, 2-aminobenzophenone). The use of this chemical strategy has some advantages over microbiological and enzymatic asymmetric synthesis protocols. In particular, the reactions can be carried out in concentrated solutions using commercially available organic solvents. The amino acids are easy to separate from the reaction mixture and the reactions are highly reproducible. Over all, it is often difficult to find enzymatic protocols for the preparation of new non-protein amino acids, because of their unusual structure.

Previously, such amino acid-Nickel complexes were incorporated in enantioselective Michael additions,⁴ Mannich reactions,⁵ Aldol reactions⁶ and S_N2 reactions.^{7,8} After functionalization, the free amino acid can be obtained by refluxing the Ni-complex in acidic methanol. Recently, (*S*)-2-aminopent-4-ynoic acid-Ni-(*S*)-BPB **1** was obtained in good yield and with >95% diastereomeric excess using the procedure described in Scheme 1.⁸ The cleavage of complex **1** in MeOH/HCl leads to (*S*)-2-aminopent-4-ynoic acid.



Scheme 1: Synthesis of **1**: *i* 1) glycine-Ni-(*S*)-BPB, Bu₄NI, NaOH (30%), 3-bromopropyne, DCE, 20 °C 2) MeONa, MeOH, 20 °C.⁸

Terminal alkynes, like (*S*)-2-aminopent-4-ynoic acid, can be functionalized via the Sonogashira cross-coupling reaction.⁹ Halogenated sp²-hybridized compounds, like alkenes or aryls, are the coupling partner, while palladium and a copper salt are used as catalysts. However, the palladium catalyzed cross-coupling of amino acids is difficult to be carried out successfully, due to interactions between palladium and the free carboxyl and amino groups. For free (*S*)-2-aminopent-4-ynoic acid, the Sonogashira reaction has been reported to be successfully only when highly reactive iodobenzenes were employed.¹⁰ Bromobenzenes can be used after protecting the carboxyl and amino groups.¹¹

In (*S*)-2-aminopent-4-ynoic acid-Ni-(*S*)-BPB **1** both the amino and carboxyl group are ligated to nickel. Therefore, we reasoned that the Sonogashira reaction with bromobenzenes should be possible. Herein, we report Sonogashira cross-coupling reactions of Ni complex **1**. The

subsequent cleavage of the complex afforded a variety of free alkynylated amino acids in good yields with high enantiomeric excess. This strategy provides a convenient approach to optically pure alkynylated amino acids.

Furthermore the obtained (*S*)-2-amino-(5)-[aryl]pent-4-ynoic acids were tested for their biological activities and were found to be highly selective inhibitors of ALR2 over ALR1.

This enzyme plays an important role in the polyol pathway of glucose metabolism. The polyol pathway of glucose metabolism is associated to the development of various diabetes complications, a global and continually growing epidemic.¹² Hyperglycemia in diabetes is believed to be the primary cause of the long term diabetes complications such as nephropathy, cataractogenesis, retinopathy, and neuropathy and many other mechanisms which include increased aldose reductase (AR)-related polyol pathway, excessive oxidative stress and increased advanced glycation end product (AGE) formation.¹³ The increase in polyol pathway flux always result to accumulation of sorbitol in the fibre of the lens, leading to an influx of water, generation of osmotic stress, and cataract formation in which cataractogenesis leads to blindness the world over in diabetic patients.^{14,15} Also, the accumulation of sorbitol and its metabolites in the nerves, retina, and kidneys which are connected to the onset and progression of long-term diabetic complications (i.e., retinopathy, neuropathy, nephropathy and vascular complications) that are caused due to poor glycemic control.¹⁶

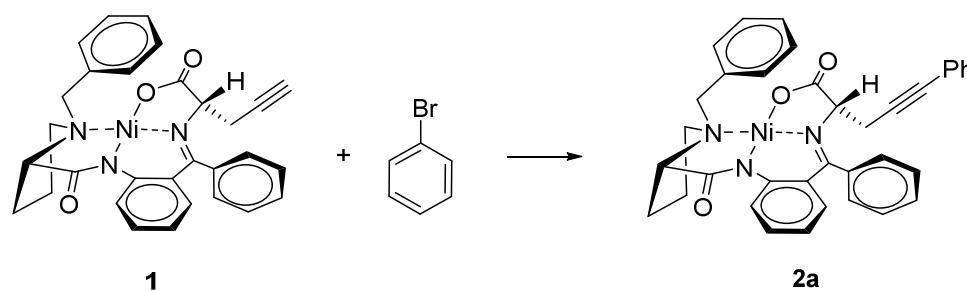
Aldehyde reductase (ALR1; EC 1.1.1.2) and aldose reductase (ALR2; EC 1.1.1.21) belong to the class of aldo-keto reductase (AKR) superfamily of enzymes whose members are believed to play critical roles in various biological functions which are associated to long-term diabetic complications.¹⁷ The two enzymes have been isolated and purified from a number of tissues including the brain, kidney, liver, lens and skeletal muscle.^{18,19} Both ALR1 and ALR2 catalyse the NADPH dependent reduction of aldehyde, xenobiotic aldehyde, ketones, trioses and triose phosphates.^{20,21} ALR1 reduces aromatic aldehyde²² while ALR2 is the first enzyme in the polyol pathway that converts glucose to sorbitol, which is subsequently transformed to fructose by sorbitol dehydrogenase. During a hyperglycaemic event, the increased in glucose level enhances ALR2 activity, increasing the glucose flux through the polyol pathway which impacts other NADPH-dependent enzymes shown to play a key role in long-term diabetic onset complications such as neuropathy and nephropathy.²² Inhibition of ALR2, thus offers patients suffering from diabetes mellitus a viable therapeutic measure against this debilitating pathologies associated with chronic hyperglycaemia.^{23,24}

Many ARIS have been reported in the literature but adverse side effects and lack of efficacy have curtailed the prospect of many drug candidates for clinical use. As a result of this, the development of potent (ALR2) inhibitors is an obvious and attractive strategy to prevent or delay the onset and progression of these complications. In this present study, different derivatives of amino acids were synthesized as selective potent inhibitors of ALR2 over ALR1.

Results and Discussion

Chemical Synthesis

Starting with the complex **1** a number of reaction conditions for the Sonogashira cross-coupling reaction were tested using bromobenzene as aryl halide (Scheme 2, Table 1).



Scheme 2: Optimization for the synthesis of **2a**.

Table 1: Optimization for the synthesis of **2a** (all experiments were carried out with 0.25 mmol of **1** in 1 ml solvent)

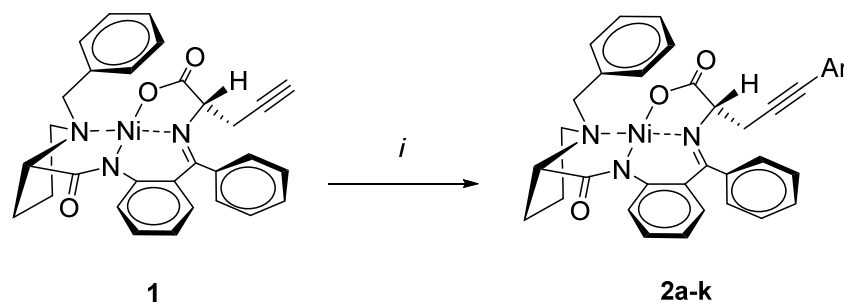
Pdsource [mol%]	CuI [mol%]	solvent	PhBr [eq.]	base [ml]	temp. [°C]	time [h]	yield [%] ^a
PdCl ₂ (PPh ₃) ₂ (5)	10	1,4-dioxane	0.84	HNiPr ₂ (0.5)	90	6	46
Pd(PPh ₃) ₄ (5)	10	1,4-dioxane	0.84	HNiPr ₂ (0.5)	90	6	70
Pd(PPh ₃) ₄ (5)	10	THF	0.84	HNiPr ₂ (0.5)	90	6	58
Pd(PPh ₃) ₄ (5)	10	DMF	0.84	HNiPr ₂ (0.5)	90	6	64
Pd(PPh ₃) ₄ (5)	10	MeCN	0.84	HNiPr ₂ (0.5)	90	6	59
Pd(PPh ₃) ₄ (5)	10	1,4-dioxane	0.84	Et ₃ N (0.5)	90	6	45
Pd(PPh ₃) ₄ (5)	5	1,4-dioxane	0.84	HNiPr ₂ (0.5)	90	6	57
Pd(PPh ₃) ₄ (2.5)	5	1,4-dioxane	0.84	HNiPr ₂	90	6	40

Pd(PPh ₃) ₄ (5)	10	DMF	0.84	HNiPr ₂ (0.5)	140	6	69
Pd(PPh ₃) ₄ (5)	10	1,4-dioxane	0.84	HNiPr ₂ (0.25)	90	6	33
Pd(PPh ₃) ₄ (5)	10	1,4-dioxane	0.84	HNiPr ₂ (1.0)	90	6	83
Pd(PPh ₃) ₄ (5)	10	1,4-dioxane	0.84	HNiPr ₂ (1.5)	90	6	71
Pd(PPh ₃) ₄ (5)	10	1,4-dioxane	1	HNiPr ₂ (1.0)	90	6	70
Pd(PPh ₃) ₄ (5)	10	1,4-dioxane	1.2	HNiPr ₂ (1.0)	90	6	79
Pd(PPh ₃) ₄ (5)	10	1,4-dioxane	1.2	HNiPr ₂ (1.0)	90	3	76

^a Isolated yield

Yields up to 83% of (*S*)-2-amino-5-phenylpent-4-ynoic acid-Ni(*S*)-BPB **2a** were obtained using 5 mol% of the inexpensive and commercially available catalyst Pd(PPh₃)₄. Attempts to reduce the amount of catalyst decreased the yield. The solvent of choice was 1,4-dioxane, while DMF, THF and acetonitrile gave slightly lower yields of **2a**. The base HNiPr₂ gave good yields, while Et₃N drastically decreased the yield. The best ratio between base and solvent proved to be 1:1. A reaction time of 3 hours was sufficient for the complete conversion of the starting material. To obtain good yields, either **1** or the bromobenzene has to be used in excess. For these reactions we decided to use commercially available bromobenzenes in excess, even if the isolated yields are slightly lower.

With the optimized reaction conditions in hand, derivatives **2a-k** were prepared (Scheme 3, Table 2).



Scheme 3: Synthesis of **2a-k**: *i*, **1** (1.0 eq.), ArBr (1.2 eq.), Pd(PPh₃)₄ (5 mol%), CuI (10 mol%), HNiPr₂ (1 ml/0.25 mmol **1**), 1,4-dioxane (1 ml/0.25 mmol **1**), 90 °C, 3 - 4.5 h.

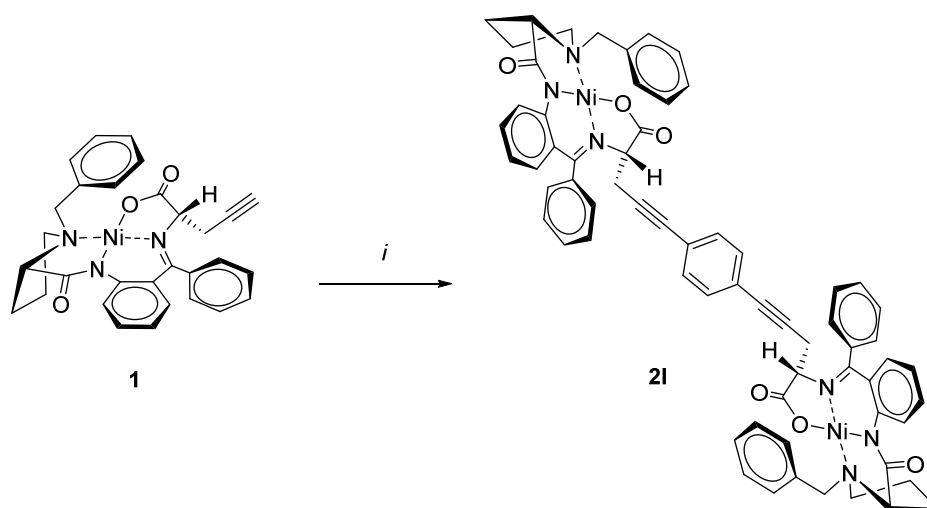
Table 2: Derivatives **2a-k**.

	Ar	time [h]	yield [%] ^a
2a	Phenyl	3.0	79
2b	4-tolyl	3.0	67
2c	3-tolyl	3.0	71
2d	2-tolyl	3.0	46
2e	naphth-1-yl	4.5	68
2f	thiophen-2-yl	3.5	78
2g	4-fluorbenzene	3.0	71
2h	4-chlorbenzene	3.0	72
2i	4-(trifluoromethyl)benzene	3.0	76
2j	3-methoxybenzene	3.5	67
2k	4-nitrobenzene	3.0	7

^a Isolated yield

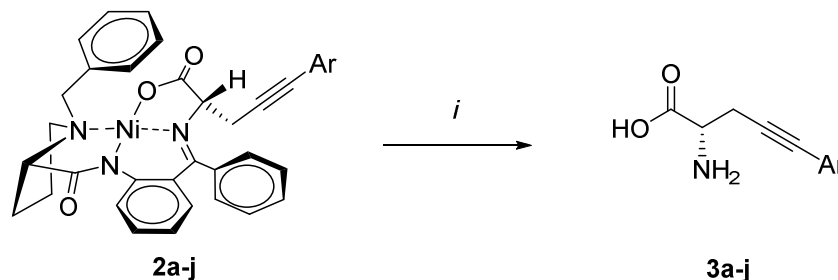
Most of the derivatives **2a-k** were obtained in good yields. The established conditions were applied for electron rich as well as electron poor bromobenzenes and gave good yields, ranging from 67 – 78 %. In case of compound **2d** only a moderate yield (46%) was achieved, which is probably due to steric effects of the methyl-group in *ortho*-position. For **2k** only 7% yield was obtained which might be explained by interactions of the nitro-group with palladium.

In addition, the twofold Sonogashira cross-coupling reaction was successfully carried out using 1,4-dibromobenzene (Scheme 4). Compound **2i** was obtained in 46% yield, which amounts to 68% for each coupling-step.

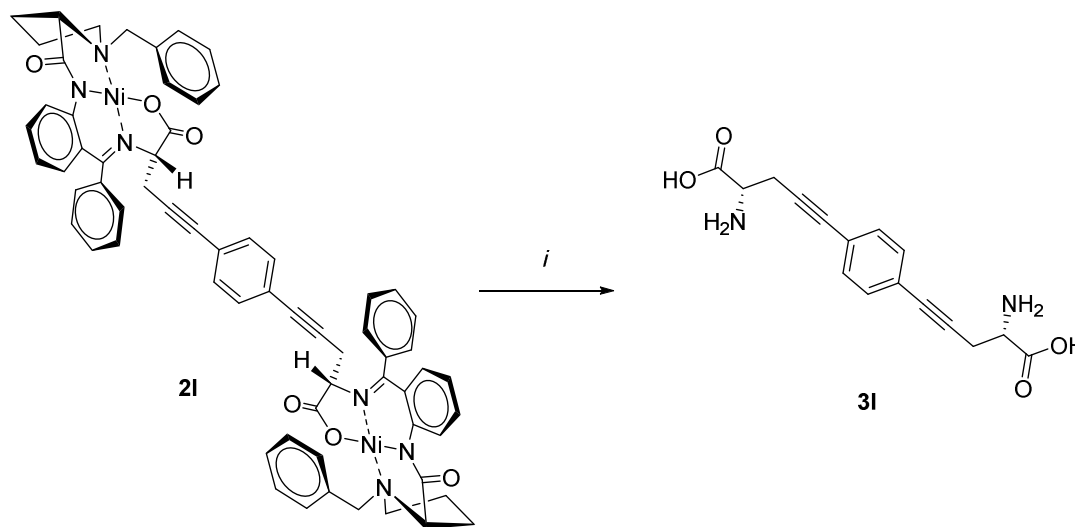


Scheme 4: Synthesis of **2l**: *i*, **1** (1.0 eq.), 1,4-Br₂C₆H₄ (0.5 eq.), Pd(PPh₃)₄ (5 mol%), CuI (10 mol%), HNiPr₂ (1 ml/0.25 mmol **1**), 1,4-dioxane (1 ml/0.25 mmol **1**), 90 °C, 4.5 h.

Subsequently, we studied the cleavage of complexes **2a-j** and **2l** to obtain the corresponding free amino acids. Compounds **2a-j** and **2l** were refluxed in a MeOH/H₂O/HCl solvent mixture for 20 minutes to give the desired free amino acids **3a-j** and **3l** in a fast and simple manner (Schemes 5 and 6, Table 3).



Scheme 5: Synthesis of **3a-j**: *i*, **2a-j** (0.4 – 1.1 mmol), MeOH (10 ml), HCl (12M, 1.5ml), H₂O (5 ml), 60 °C, 20 min.



Scheme 6: Synthesis of **3l**: *i*, **2l** (0.5 mmol), MeOH (10 ml), HCl (12M, 1.5ml), H₂O (5 ml), 60 °C, 20 min.

Table 3: Derivatives **3a-j** and **3l**.

	Ar	yield [%] ^a
3a	Phenyl	87
3b	4-tolyl	89
3c	3-tolyl	86
3d	2-tolyl	72

3e	naphth-1-yl	52
3f	thiophen-2-yl	88
3g	4-fluorbenzene	90
3h	4-chlorbenzene	84
3i	4-(trifluoromethyl)benzene	92
3j	3-methoxybenzene	99
3l	1,4-benzene	85

^a Isolated yield

The free amino acids **3a-j** and **3l** were obtained in very good to excellent yields. Electron donating and electron withdrawing groups on the benzene ring were not affecting the yield. Compound **3e** resulted in lower yield caused by difficulties during the work up process via cation-exchange column due to its low water solubility. Finally the enantiomeric excess of **3g** and **3j** was checked by chiral HPLC (CrownpakCR(-) column). The enantiomeric excess of the amino acids **3g** and **3j** were estimated as >99%. This proves that the optical purity is not effected by the Sonogashira cross-coupling reaction or the cleavage step.

OSIRIS Drug Properties and Toxicity Profile

All of the newly synthesized compounds were found in good agreement with Lipinski's rule of five²⁵ as calculated by OSIRIS property explorer.²⁶ The overall OSIRIS drug score of compounds was in the range of 4.5 - 4.7, which showed that they were very good drug candidates. However, there is an exemption of compound **3e**, which exhibited 2.5 OSIRIS drug score. When the compounds were screened for drug toxicity on tumorigenic, mutagenic, irritability and reproductive toxicity they were found non-toxic. However, compound **3e** showed a mild tumorigenic toxicity due to the presence of naph-1-yl substitution.

Biological Tests

ALR1 inhibitory activity

Aldehyde reductase is a cytosolic enzyme of an aldo-keto reductase superfamily member like aldose reductase (ALR2). It also catalyzes different physiological functions, among them, is detoxification of toxic aldehydes.^{27,28,29} More so, these enzymes share a high level of sequence of about 65% and structural homology³⁰ as well as chemical and physical characteristics.²⁷ This increased tendency of a lot of substrates or ligands to bind to them.²⁹ In this study, two different derivatives of amino acids (phenyl and aliphatic) series were examined on purified ALR1 from bovine kidney to determine the one that would be a

selective inhibitor of ALR2 over ALR1 as a potent inhibitor for future treatment of diabetic complications, expressed as IC_{50} values followed by their pIC_{50} ($-\log IC_{50}$) presented in Table 4. The results obtained from this study were presented on Table 4 and it was observed that among all the tested compounds, compounds **3h**, **3i**, and **3d** found to be strongly inhibited by ALR1 as compared to the other compounds which are potent inhibitors of ALR2 with the IC_{50} values of 0.041 ± 0.08 , 0.065 ± 0.07 and 0.09 ± 0.01 respectively. The chlorine substituted derivative compound **3h** (IC_{50} 0.041 ± 0.08) was found to be inhibiting ALR1 with high IC_{50} value.

Finally, it was observed that increase in the electro negativity groups on different derivatives further decrease the inhibitory activity of these tested compounds against ALR1, therefore this could be a hint and re-examined critically in future as it could be a search light for the synthesis of high selective inhibitor of aldose reductase (ARIS).

ALR2 inhibitory activity

ALR2 inhibitors, many of which were potent both *in vitro* and *in vivo* experimental evaluations using animals, but on clinical trials they were failed which could be as a result of their side effects or poor efficacy. These side effects may be due to their failures to selective inhibit the ALR2 with respect to ALR1 (aldehyde reductase).³¹ One of the most important attributes of pharmacological relevance of ARIs is their high selectivity over other members of the aldo-keto reductase family while the simultaneous inhibition of other structural related physiological important oxido-reductases may create undesirable effects but to ascertain the selectivity of the aldose reductase (ALR2), the closely related aldehyde reductase (ALR1) is always studied along.³² In sequence, ALR1 and ALR2 similarity is close to about 65%.³³ In this present study, ALR 2 was selective studied over ALR1 using a purified calf lenses.

The inhibitory activity of aldose reductase was conducted on different derivatives of phenyl and aliphatic amino acids series at 1 mM concentration and compound having percentage inhibition greater than 50% was further diluted to the concentrations of eight to produce dose-response curve for determination of IC_{50} value which was basically the monitoring of NADPH oxidation by spectrophotometric method which is known to be a reliable standard method³⁰ and quercetin was used as the standard control. All the compounds tested on this enzyme shown to be highly selective potent inhibitors and their inhibitory activities were at the micro molar level as to the value obtained for standard inhibitor.

As shown in Table 4, the most active/potent compounds among the tested compounds against ALR2 with the high inhibitory activities were compounds, **3e**, **3i** and **3l** with the IC_{50} values of 0.012 ± 0.09 , 0.068 ± 0.01 , 0.078 ± 0.001 and 0.10 ± 0.03 with the pIC_{50} values of 7.9, 7.2, 7.1 and 7.0 respectively. The value of pIC_{50} is a good indicator to determine the potent inhibitor, because the higher its value the more potent the inhibitor. The least potent compounds as compared to the other compounds which show least inhibitory activity was compound **3c** with the IC_{50} value of 4.32 ± 0.04 by compounds **3h** with the IC_{50} values of 3.29 ± 0.20 . The other tested amino acids were also promising inhibitors of the enzyme ALR2 and their pIC_{50} values are in the range of 5.9 - 6.9 as presented in Table 4.

Generally, in this series of compounds, the unsubstituted phenyl (compound **3a**) was the most potent inhibitor of ALR2. Finally, the strong inhibitory potency of this compound indicates that the presence of an additional groups on it may not be a prerequisite feature for ARIs activity, the potency and selectivity of this compounds as compared to the standard control and ALR1 are worthy of further development.

Table 4: The IC_{50} and pIC_{50} values for ALR1 and ALR2 activities against amino acids **3a-j** and **3l**.

Aryl-Rest	$IC_{50}^{ALR1} \pm SEM^a$ [μM]	pIC_{50}	$IC_{50}^{ALR2} \pm SEM^a$ [μM]	pIC_{50}
3a Phenyl	1.05±0.03	6.0	0.012±0.09	7.9
3b 4-tolyl	0.13±0.05	6.8	1.10±0.28	5.9
3c 3-tolyl	5.20±0.17	5.3	4.32±0.04	5.3
3d 2-tolyl	0.09±0.01	7.0	0.45±0.18	6.3
3e naphth-1-yl	0.40±0.02	6.4	0.068±0.001	7.2
3f thiophen-2-yl	0.60±0.10	6.22	0.37±0.08	6.4
3g 4-fluorbenzene	1.53±0.02	5.8	0.13±0.02	6.9
3h 4-chlorbenzene	0.041±0.08	7.4	3.29±0.20	5.5
3i 4-(trifluoromethyl)benzene	0.065±0.07	7.2	0.078±0.001	7.1
3j 3-methoxybenzene	0.14±0.05	6.9	0.17±0.02	6.8
3l 1,4-benzene	1.05±0.03	6.0	0.10±0.03	7
Valproic acid	56.01±0.59	4.3	---	--
Quercetin	---	----	3.04±0.50	5.51

^a $n = 3$; --- not determined

Molecular Docking

As evident from the in-vitro results compound **3a** was the most suitable compound for docking studies because of its potent and selective nature toward the ALR-2 enzyme. Molecular docking of compound **3a** was carried out after successfully reproducing poses similar to the reference ligand pose. The deviation (RMSD value) from the co-crystallized reference ligand poses were around 1.89 Å and 0.69 Å in case of ALR-1 and ALR-2, respectively. After reproducing the reference ligand poses, the compound **3a** was docked inside the active site.

The molecular docking of compound **3a** revealed perfect orientation of the test compound inside the ALR-2 as compared to ALR-1. The compound forms perfect orientation inside the ALR-2 active site by forming hydrogen bonding interactions with catalytic amino acid residues of Tyr48, His110 and Trp111. The benzene ring of the compound **3a** is perfectly oriented inside the specificity determining pocket of ALR-2 formed by amino acids Tyr111, Thr113, Phe122, Ala299 and Leu300 as described previously.³⁴ Furthermore, the compound forms Pi-Pi stacking interaction with amino acid Trp111. Amino acid Trp20 was also found to form Pi - charge interactions with compound, which further stabilizes the pose. The HYDE assessment of the compound inside the ALR-2 shows that the compound binds to the receptor with a very high binding affinity (i.e. -32 KJmol⁻¹) as compared to its binding affinity inside the active pocket of ALR-1 (i.e. -17 KJmol⁻¹).

Inside ALR-1 the compound **3a** forms hydrogen bonding interactions with amino acid Trp114 and Arg312. Such hydrogen bonding interactions are similar to the previously reported interactions formed by 3,5-dichlorosalicylic acid.³⁵ However, hydrogen bonding interactions previously reported³⁵ with amino acid Trp22, Tyr50 and His113 have not been observed. Furthermore, Inside ALR-1 the compound was also found to have weak hydrophobic interaction with amino acid Trp22 and amino acid His113 was found to form Pi-charge interaction with our compound. The difference in binding affinity of our compound **3a** against ALR-1 and ALR-2 enzymes calculated by HYDE assessment may be attributed to differences in IC₅₀ value against such enzymes. The putative binding mode of compound **3a** can be seen in Figure 1.

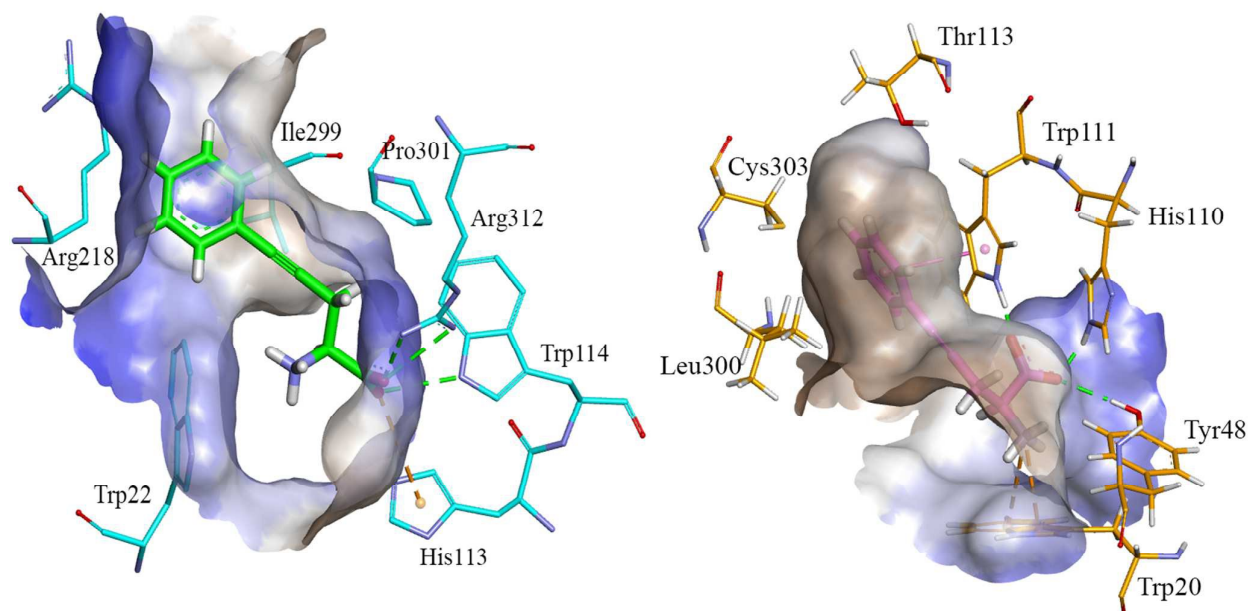


Fig. 1 Putative binding mode of Compound **3a** inside active site of porcine ALR-1 (coloured cyan) and inside active site of human ALR-2 (coloured brown).

Conclusion

We have described a straight forward strategy to prepare a variety of optically pure (*S*)-2-amino-5-arylpent-4-ynoic acids (alkynylated amino acids) with a broad preparative scope. Substrates and catalysts are readily available. The synthesized amino acids were tested for biological activity and were found to be highly selective inhibitors of ALR2 over ALR1 and were confirmed by molecular docking study. This enzyme plays an important role in the polyol pathway of glucose metabolism and is associated to the development of various diabetes complications.

Experimental section

Chemical synthesis

The Sonogashira reaction was performed in anhydrous solvents (DMF, THF, dioxane, MeCN) under argon using a vacuum-line and standard Schlenk techniques. Ethyl acetate, heptane and dichloromethane were distilled before usage. All other solvents and reagents were of reagent grade and used as received. For column chromatography Fluka silica gel 60 (0.063-0.200 mm, 70-320 mesh) was used. For the cation exchange column dowex 50WX8 H⁺ was used. NMR spectra were recorded with Bruker AV 500 (75 MHz), Bruker AV 300 III (62.9 MHz) and Bruker AV 250 II (62.9 MHz) spectrometers and are reported in ppm. The NMR spectra are calibrated by solvent at 7.26 (CDCl₃), 3.31 (D₃COD) 4.79 (D₂O) for ¹H and 77.23 (CDCl₃), 49.15(D₃COD) for ¹³C. MS and HRMS spectra were recorded with a Finnigan MAT 95 XP spectrometer. IR spectra were recorded with a Nicolet 6700 FT-IR spectrometer. EA spectra were recorded with a Flash EA 112 Series. Specific rotations were measured with a Gyromat-HP, 20 mm cell. Melting points were measured with a Micro-Hot-Stage GalenTM III Cambridge Instruments.

General procedure for 2a-l: (*S*)-2-aminopent-4-ynoic acid-Ni-(*S*)-BPB **1** (1.0 eq.) was added to a stirred solution of Pd(PPh₃)₄ (5 mol%), CuI (10 mol%), bromobenzene (1.2 eq. (0.5 eq. for **2l**)), HNiPr₂ (4 ml/mmol **1**) and 1,4-dioxane (4 ml/mmol **1**). The mixture was stirred for 3 – 4.5 h at 90 °C. After cooling to room temperature the mixture was diluted with ethyl acetate and washed two times with water. The organic layer was dried with Na₂SO₄ and filtered. Afterwards the solvent was removed under reduced pressure and the residue was purified by column chromatography (EE/Hep, 5:1) to obtain **2a-l** as a red solid.

(*S*)-2-Amino-5-[phenyl]pent-4-ynoic acid-Ni-(*S*)-BPB (2a**):** Reaction of **1** (134 mg, 0.250 mmol), Pd(PPh₃)₄ (14.4 mg, 0.013 mmol), CuI (4.8 mg, 0.025 mmol) and bromobenzene (31.6 μl, 0.300 mmol) in HNiPr₂ (1ml) and 1,4-dioxane (1ml) for 3 h gave **2a** as a red solid (116 mg, 76%); mp 126-128 °C. ¹H NMR (300 MHz, CDCl₃): δ = 1.53–1.67 (m, 1H, CH_{2,Pro}), 1.98–2.14 (m, 3H, CH_{2,Pro}), 2.52 (dd, ³J = 6.4 Hz, ²J = 17.0 Hz, 1H, CH_{2,Gly}), 2.93 (d, ²J = 17.0 Hz, 1H, CH_{2,Gly}), 3.04–3.20 (m, 1H, CH_{2,Pro}), 3.36 (pt, ³J = 8.3 Hz, 1H, CH_{Pro}), 3.56 (d, ²J = 12.6 Hz, 1H, CH₂Ph), 3.59–3.71 (m, 1H, CH_{2,Pro}), 4.12 (d, ³J = 6.4 Hz, 1H, CH_{Gly}), 4.42 (d, ²J = 12.6 Hz, 1H, CH₂Ph), 6.70 (d, *J* = 3.9 Hz, 2H, C₆H₄), 7.11 (d, *J* = 7.3 Hz, 1H, Ar), 7.16–7.25 (m, 2H, Ar), 7.27–7.40 (m, 6H, Ar), 7.46–7.60 (m, 5H, Ar), 8.09 (d, *J* = 7.3 Hz, 2H, C₆H₅), 8.26 (d, *J* = 8.6 Hz, 1H, C₆H₄). ¹³C NMR(75 MHz, CDCl₃): δ = 23.3, 24.5, 30.3, 57.5, 63.3 (CH₂), 70.1 (CH), 84.4, 85.7 (C ≡ C), 120.5, 123.7, 126.7, 127.6, 128.3, 128.4,

128.7, 128.8, 129.0, 129.8, 131.5, 132.1, 132.4, 133.3 (CH), 123.2, 126.1, 133.4, 134.1, 142.9 (C_{Ar}), 171.8, 180.5 (C). IR (ATR, cm⁻¹): $\tilde{\nu}$ = 3436 (br, w), 2954 (w), 1671 (s), 1636 (s), 1585 (m), 1541 (m), 1438 (m), 1337 (s), 1254 (s), 1164 (m), 1064 (w), 922 (w), 752 (s), 701 (s), 540 (m). MS (EI, 70 eV): m/z (%) = 611 (M⁺, 34), 567 (25), 476 (8), 380 (8), 164 (6), 217 (56), 160 (100), 91 (98). HRMS (ESI): calcd for C₃₆H₃₂N₃NiO₃ ([M+H]⁺) 612.17917, found 612.17906, calcd for C₃₆H₃₁N₃NaNiO₃ ([M+Na]⁺) 634.16111, found 634.16131.

(S)-2-Amino-5-[4-tolyl]pent-4-ynoic acid-Ni-(S)-BPB (2b): Reaction of **1** (804 mg, 1.5 mmol), Pd(PPh₃)₄ (86.0 mg, 0.075 mmol), CuI (28.6 mg, 0.150 mmol) and 4-bromotoluene (307.9 mg, 1.8 mmol) in HNiPr₂ (6 ml) and 1,4-dioxane (6 ml) for 3 h gave **2b** as a red solid (614 mg, 66%); mp 218–219 °C. ¹H NMR (300 MHz, CDCl₃): δ = 1.51–1.66 (m, 1H, CH_{2,Pro}), 1.94–2.12 (m, 3H, CH_{2,Pro}), 2.33 (s, 3H, CH₃), 2.48 (dd, ³J = 6.7 Hz, ²J = 17.2 Hz, 1H, CH_{2,Gly}), 2.90 (dd, ³J = 2.7 Hz, ²J = 17.2 Hz, 1H, CH_{2,Gly}), 3.04–3.19 (m, 1H, CH_{2,Pro}), 3.33 (pt, ³J = 8.6 Hz, 1H, CH_{Pro}), 3.54 (d, ²J = 12.6 Hz, 1H, CH_{2Ph}), 3.55–3.65 (m, 1H, CH_{2,Pro}), 4.09 (dd, ³J = 2.7 Hz, ³J = 6.7 Hz, 1H, CH_{Gly}), 4.42 (d, ²J = 12.6 Hz, 1H, CH_{2Ph}), 6.68 (d, J = 4.0 Hz, 2H, C₆H₄), 7.05–7.56 (m, 13H, Ar), 8.06 (d, J = 7.2 Hz, 2H, C₆H₅), 8.24 (d, J = 8.7 Hz, 1H, C₆H₄). ¹³C NMR (75 MHz, CDCl₃): δ = 21.7 (CH₃), 23.6, 24.8, 30.6, 57.7, 63.5 (CH₂), 68.3, 70.4 (CH), 83.8, 86.1 (C \equiv C), 120.7, 123.9, 127.0, 128.0, 129.0, 129.0, 129.3, 129.3, 129.4, 130.1, 131.8, 132.2, 132.6, 133.6, (CH_{Ar}), 120.4, 126.4, 133.6, 134.4, 138.6, 143.2 (C_{Ar}), 172.0, 179.2, 180.8 (C). IR (ATR, cm⁻¹): $\tilde{\nu}$ = 3054 (br, w), 3007 (br, w), 2871 (br, w), 1675 (s), 1620 (s), 1585 (m), 1543 (w), 1435 (w), 1337 (s), 1258 (s), 1162 (m), 1085 (w), 1029 (w), 860 (w), 828 (m), 774 (m), 756 (s), 747 (s), 699 (s), 629 (w), 567 (w). MS (EI, 70 eV): m/z (%) = 625 (M⁺, 34), 581 (25), 490 (7), 394 (10), 251 (9), 217 (46), 205 (9), 160 (86), 91 (100). HRMS (ESI): calcd for C₃₇H₃₄N₃NiO₃ ([M+H]⁺) 626.19482, found 626.19498, calcd for C₃₇H₃₃N₃NaNiO₃ ([M+Na]⁺) 648.17676, found 648.17663.

(S)-2-Amino-5-[3-tolyl]pent-4-ynoic acid-Ni-(S)-BPB (2c): Reaction of **1** (804 mg, 1.5 mmol), Pd(PPh₃)₄ (86.0 mg, 0.075 mmol), CuI (28.6 mg, 0.150 mmol) and 3-bromotoluene (219.0 μ l, 1.8 mmol) in HNiPr₂ (6 ml) and 1,4-dioxane (6 ml) for 3 h gave **2c** as a red solid (653 mg, 71%); mp 115–116 °C. ¹H NMR (300 MHz, CDCl₃): δ = 1.51–1.62 (m, 1H, CH_{2,Pro}), 1.95–2.09 (m, 3H, CH_{2,Pro}), 2.27 (s, 3H, CH₃), 2.48 (dd, ³J = 6.8 Hz, ²J = 17.2 Hz, 1H, CH_{2,Gly}), 2.89 (dd, ³J = 2.7 Hz, ²J = 17.2 Hz, 1H, CH_{2,Gly}), 3.01–3.15 (m, 1H, CH_{2,Pro}), 3.33 (pt, ³J = 8.4 Hz, 1H, CH_{Pro}), 3.51 (d, ²J = 12.6 Hz, 1H, CH_{2Ph}), 3.55–3.64 (m, 1H, CH_{2,Pro}), 4.09 (dd, ³J = 6.8 Hz, ³J = 2.7 Hz, 1H, CH_{Gly}), 4.38 (d, ²J = 12.6 Hz, 1H, CH_{2Ph}), 6.63–6.68 (m, 2H, C₆H₄), 7.06–7.54 (m, 13H, Ar), 8.06 (d, J = 7.1 Hz, 2H, C₆H₅), 8.25 (d, J = 8.6 Hz,

1H, C₆H₄). ¹³C NMR(75 MHz, CDCl₃): δ = 21.3 (CH₃), 23.5, 24.7, 30.4, 57.7, 63.5 (CH₂), 70.3 (CH), 84.1, 86.1 (C ≡ C), 120.6, 123.9, 126.9, 127.9, 128.4, 128.9, 129.0, 129.2, 129.4, 130.0, 131.7, 132.5, 132.9, 133.5 (CH_{Ar}), 123.1, 126.3, 134.4, 138.3, 143.1 (C_{Ar}), 171.9, 179.1, 180.7 (C). IR (ATR, cm⁻¹): $\tilde{\nu}$ = 3057 (br, w), 3027 (br, w), 2952 (br, w), 2869 (br, w), 1732 (w), 1671 (s), 1634 (s), 1585 (m), 1541 (m), 1438 (m), 1363 (m), 1336 (s), 1310 (m), 1253 (s), 1164 (s), 1063 (w), 882 (w), 750 (s), 701 (s), 625 (m), 567 (m), 540 (m). MS (EI, 70 eV): *m/z* (%) = 625 (M⁺, 78), 581 (39), 490 (12), 424 (7), 394 (14), 264 (10), 219 (32), 217 (85) 205 (10), 160 (100), 129 (8), 91 (93), 44 (9). HRMS (EI): calcd for C₃₇H₃₃N₃NiO₃ ([M]) 625.18699, found 625.18724.

(S)-2-Amino-5-[2-tolyl]pent-4-ynoic acid-Ni-(S)-BPB (2d): Reaction of **1** (804 mg, 1.5 mmol), Pd(PPh₃)₄ (86.0 mg, 0.075 mmol), CuI (28.6 mg, 0.150 mmol) and 2-bromotoluene (219.0 μl, 1.8 mmol) in HNiPr₂ (6 ml) and 1,4-dioxane (6 ml) for 3 h gave **2d** as a red solid (427 mg, 46%); mp 107-108 °C. ¹H NMR (250 MHz, CDCl₃): δ = 1.52–1.63 (m, 1H, CH_{2,Pro}), 1.73–1.86 (m, 1H, CH_{2,Pro}), 1.92–2.05 (m, 2H, CH_{2,Pro}), 2.32 (s, 3H, CH₃), 2.52 (dd, ³*J* = 7.0 Hz, ²*J* = 17.1 Hz, 1H, CH_{2,Gly}), 2.92 (dd, ³*J* = 2.2 Hz, ²*J* = 17.1 Hz, 1H, CH_{2,Gly}), 2.98–3.10 (m, 1H, CH_{2,Pro}), 3.30 (dd, ³*J* = 6.4 Hz, ³*J* = 10.5 Hz, 1H, CH_{Pro}), 3.50 (d, ²*J* = 12.6 Hz, 1H, CH₂Ph), 3.53–3.63 (m, 1H, CH_{2,Pro}), 4.11 (d, ³*J* = 7.0 Hz, 1H, CH_{Gly}), 4.38 (d, ²*J* = 12.6 Hz, 1H, CH₂Ph), 6.67 (d, *J* = 3.4 Hz, 2H, C₆H₄), 7.03–7.57 (m, 13H, Ar), 8.06 (d, *J* = 7.3 Hz, 2H, C₆H₅), 8.22 (d, *J* = 8.6 Hz, 1H, C₆H₄). ¹³C NMR(62.9 MHz, CDCl₃): δ = 20.7 (CH₃), 23.5, 24.7, 30.4, 57.8, 63.5 (CH₂), 70.2 (CH), 84.8, 88.4 (C ≡ C), 120.6, 123.9, 125.8, 126.9, 127.8, 128.6, 128.9, 129.0, 129.2, 129.7, 130.1, 131.8, 132.5, 133.5, 133.6 (CH_{Ar}), 123.1, 126.2, 134.4, 140.9, 143.2 (C_{Ar}), 171.9, 179.2, 180.8 (C). IR (ATR, cm⁻¹): $\tilde{\nu}$ = 3059 (br, w), 2952 (br, w), 2870 (br, w), 1672 (s), 1635 (s), 1585 (m), 1438 (m), 1363 (m), 1337 (s), 1254 (s), 1164 (m), 1074 (br, w), 752 (s), 701 (s), 540 (m). MS (EI, 70 eV): *m/z* (%) = 625 (M⁺, 20), 583 (14), 581 (13), 490 (5), 394 (5), 219 (14), 217 (37), 165 (8), 160 (86), 128 (7), 91 (100), 44 (19). HRMS (EI): calcd for C₃₇H₃₃N₃NiO₃ ([M]) 625.18699, found 625.18781.

(S)-2-Amino-5-[naphth-1-yl]pent-4-ynoic acid-Ni-(S)-BPB (2e): Reaction of **1** (804 mg, 1.5 mmol), Pd(PPh₃)₄ (86.0 mg, 0.075 mmol), CuI (28.6 mg, 0.150 mmol) and 1-bromonaphthalene (259.0 μl, 1.8 mmol) in HNiPr₂ (6 ml) and 1,4-dioxane (6 ml) for 4.5 h gave **2e** as a red solid (676 mg, 68%); mp 185-186 °C. ¹H NMR (300 MHz, CDCl₃): δ = 1.36–1.52 (m, 1H, CH_{2,Pro}), 1.62–1.87 (m, 2H, CH_{2,Pro}), 1.93–2.03 (m, 1H, CH_{2,Pro}), 2.52–2.72 (m, 2H, CH_{2,Gly}), 2.98–3.04 (m, 1H, CH_{2,Pro}), 3.08–3.21 (m, 1H, CH_{Pro}), 3.36 (d, ²*J* = 12.5 Hz, 1H, CH₂Ph), 3.39–3.54 (m, 1H, CH_{2,Pro}), 3.99–4.20 (m, 1H, CH_{Gly}), 4.29 (d, ²*J* =

12.5 Hz, 1H, CH₂Ph), 6.54–6.77 (m, 2H, C₆H₄), 7.01–7.77 (m, 15H, Ar), 7.98 (d, *J* = 7.5 Hz, 2H, C₆H₅), 8.22–8.29 (m, 2H, C₆H₄). ¹³C NMR(62.9 MHz, CDCl₃): δ = 23.3, 25.1, 30.2, 57.9, 63.5 (CH₂), 70.2 (CH), 84.0, 89.5 (C ≡ C), 120.7, 121.0, 124.1, 125.4, 126.2, 126.3, 126.7, 126.9, 127.3, 127.9, 128.3, 128.9, 129.0, 129.3, 130.1, 131.1, 131.8, 132.7, 133.3, 133.6, 133.7, 133.7, 134.5, 143.4 (C_{Ar}/CH_{Ar}), 172.2, 179.3, 180.8 (C). IR (ATR, cm⁻¹): $\tilde{\nu}$ = 3057 (br, w), 2953 (br, w), 2869 (br, w), 1732 (w), 1672 (m), 1634 (s), 1584 (m), 1438 (m), 1336 (s), 1253 (s), 1164 (m), 1063 (br, w), 802 (m), 774 (m), 750 (s), 700 (s), 567 (m), 539 (m). MS (EI, 70 eV): *m/z* (%) = 661 (M⁺, 70), 617 (31), 526 (7), 264 (9), 217 (48), 165 (25) 160 (100), 91 (85). HRMS (EI): calcd for C₄₀H₃₃N₃NiO₃ ([M]⁺) 661.18699, found 661.18873.

(S)-2-Amino-5-[thiophen-2-yl]pent-4-ynoic acid-Ni-(S)-BPB (2f): Reaction of **1** (804 mg, 1.5 mmol), Pd(PPh₃)₄ (86.0 mg, 0.075 mmol), CuI (28.6 mg, 0.150 mmol) and 2-bromothiophene (174.3 μl, 1.8 mmol) in HNiPr₂ (6 ml) and 1,4-dioxane (6 ml) for 3.5 h gave **2f** as a red solid (716 mg, 78%); mp 123–124 °C. ¹H NMR (300 MHz, CDCl₃): δ = 1.61–1.76 (m, 1H, CH_{2,Pro}), 2.02–2.25 (m, 3H, CH_{2,Pro}), 2.52 (dd, ³*J* = 6.6 Hz, ²*J* = 17.3 Hz, 1H, CH_{2,Gly}), 2.94 (dd, ³*J* = 2.3 Hz, ²*J* = 17.3 Hz, 1H, CH_{2,Gly}), 3.11–3.25 (m, 1H, CH_{2,Pro}), 3.37 (pt, ³*J* = 8.0 Hz, 1H, CH_{Pro}), 3.56 (d, ²*J* = 12.6 Hz, 1H, CH₂Ph), 3.59–3.66 (m, 1H, CH_{2,Pro}), 4.05–4.12 (m, 1H, CH_{Gly}), 4.40 (d, ²*J* = 12.6 Hz, 1H, CH₂Ph), 6.67 (d, *J* = 4.0 Hz, 2H, C₆H₄), 6.97 (pt, *J* = 4.5 Hz, 1H, Ar), 7.09 (d, *J* = 6.7 Hz, 1H, Ar), 7.15–7.58 (m, 10H, Ar), 8.06 (d, *J* = 7.3 Hz, 2H, C₆H₅), 8.24 (d, *J* = 8.5 Hz, 1H, C₆H₄). ¹³C NMR(62.9 MHz, CDCl₃): δ = 23.5, 24.8, 30.5, 57.5, 63.3 (CH₂), 67.8, 70.2 (CH), 88.5 (C ≡ C), 120.6, 123.7, 126.7, 127.0, 127.2, 127.7, 128.8, 129.1, 129.9, 131.6, 132.5, 132.6, 133.4 (CH_{Ar}), 123.2, 126.2, 133.3, 134.1, 143.0 (C_{Ar}), 171.9, 178.8, 180.5 (C). IR (ATR, cm⁻¹): $\tilde{\nu}$ = 3061 (br, w), 2954 (br, w), 2858 (br, w), 1731 (w), 1672 (s), 1633 (s), 1585 (m), 1438 (m), 1364 (m), 1336 (s), 1253 (s), 1164 (s), 750 (s), 700 (s), 625 (m), 568 (m), 538 (m). MS (EI, 70 eV): *m/z* (%) = 617 (M⁺, 37), 573 (18), 217 (34), 160 (100), 91 (85). HRMS (EI): calcd for C₃₄H₃₀N₃NiO₃S ([M+H]⁺) 618.13559, found 618.13638, calcd for C₃₄H₂₉N₃NaNiO₃S ([M+Na]⁺) 640.11753, found 640.11793.

(S)-2-Amino-5-[4-fluorobenzene]pent-4-ynoic acid-Ni-(S)-BPB (2g): Reaction of **1** (804 mg, 1.5 mmol), Pd(PPh₃)₄ (86.0 mg, 0.075 mmol), CuI (28.6 mg, 0.150 mmol) and 1-bromo-4-fluorobenzene (196.4 μl, 1.8 mmol) in HNiPr₂ (6 ml) and 1,4-dioxane (6 ml) for 3 h gave **2g** as a red solid (665 mg, 71%); mp 118 °C. ¹H NMR (300 MHz, CDCl₃): δ = 1.53–1.69 (m, 1H, CH_{2,Pro}), 1.95–2.16 (m, 3H, CH_{2,Pro}), 2.49 (dd, ³*J* = 6.8 Hz, ²*J* = 17.2 Hz, 1H, CH_{2,Gly}), 2.88 (dd, ³*J* = 2.4 Hz, ²*J* = 17.2 Hz, 1H, CH_{2,Gly}), 3.07–3.22 (m, 1H, CH_{2,Pro}), 3.36 (pt, ³*J* = 8.3 Hz, 1H, CH_{Pro}), 3.48–3.63 (m, 2H, CH₂Ph/CH_{2,Pro}), 4.03–4.16 (m, 2H, CH_{2,Pro}/CH_{Gly}), 4.39

(d, $^2J = 12.6$ Hz, 1H, CH₂Ph), 6.67 (d, $J = 4.1$ Hz, 2H, C₆H₄), 6.95–7.08 (m, 3H, Ar), 7.13–7.22 (m, 2H, Ar), 7.25–7.38 (m, 3H, Ar), 7.42–7.56 (m, 5H, Ar), 8.05 (d, $J = 7.4$ Hz, 2H, C₆H₅), 8.22 (d, $J = 8.7$ Hz, 1H, C₆H₄). ¹³C NMR(75 MHz, CDCl₃): $\delta = 23.5, 24.7, 30.5, 57.6, 60.5$ (CH₂), 63.6, 70.3 (CH), 84.4, 84.8 (C \equiv C), 115.9 (d, $^2J = 22.2$ Hz, CH_{FPh}), 119.5 (d, $^4J = 3.3$ Hz, C_{FPh}), 120.8, 123.9 (CH_{Ar}), 126.3 (C_{Ar}), 126.9, 127.9, 129.0, 129.3, 130.1, 131.7, 132.7, 133.6 (CH_{Ar}), 133.5 (C_{Ar}), 134.2 (d, $^3J = 8.2$ Hz, CH_{FPh}), 134.3, 143.1 (C_{Ar}), 162.7 (d, $^1J = 249$ Hz, C_{FPh}), 172.0, 179.1, 180.8 (C). ¹⁹F NMR (282.4 MHz, CDCl₃): $\delta = -110.6$. IR (ATR, cm⁻¹): $\tilde{\nu} = 3058$ (br, w), 2692 (br, w), 2868 (br, w), 1732 (w), 1670 (s), 1632 (s), 1585 (m), 1505 (s), 1438 (m), 1364 (m), 1335 (s), 1254 (s), 1219 (m), 1164 (m), 1063 (m), 836 (m), 750 (s), 700 (s), 625 (m), 537 (m). MS (EI, 70 eV): m/z (%) = 629 (M⁺, 43), 585 (39), 494 (10), 398 (9), 217 (77), 160 (100), 91 (100), 44 (22). HRMS (ESI): calcd for C₃₆H₃₁FN₃NiO₃ ([M+H]⁺) 630.16974, found 630.17039, calcd for C₃₆H₃₀N₃NaNiO₃ ([M+Na]⁺) 652.15169, found 652.15267.

(S)-2-Amino-5-[4-chlorobenzene]pent-4-ynoic acid-Ni-(S)-BPB (2h): Reaction of **1** (804 mg, 1.5 mmol), Pd(PPh₃)₄ (86.0 mg, 0.075 mmol), CuI (28.6 mg, 0.150 mmol) and 1-bromo-4-chlorobenzene (344.7 mg, 1.8 mmol) in HNiPr₂ (6 ml) and 1,4-dioxane (6 ml) for 3 h gave **2h** as a red solid (693 mg, 72%); mp 133-134 °C. ¹H NMR (300 MHz, CDCl₃): $\delta = 1.50$ – 1.65 (m, 1H, CH_{2,Pro}), 1.88–2.13 (m, 3H, CH_{2,Pro}), 2.37–2.51 (m, 1H, CH_{2,Gly}), 2.83 (dd, $^3J = 3.7$ Hz, $^2J = 17.0$ Hz, 1H, CH_{2,Gly}), 3.02–3.18 (m, 1H, CH_{2,Pro}), 3.30 (d, $^3J = 8.1$ Hz, 1H, CH_{Pro}), 3.42–3.55 (m, 2H, CH₂Ph/CH_{2,Pro}), 3.97–4.10 (m, 1H, CH_{Gly}), 4.26–4.38 (m, 1H, CH₂Ph), 6.54–6.66 (m, 2H, C₆H₄), 6.96–7.58 (m, 13H, Ar), 7.93–8.05 (m, 2H, C₆H₅), 8.11–8.21 (m, 1H, C₆H₄). ¹³C NMR(75 MHz, CDCl₃): $\delta = 23.6, 24.7, 30.5, 57.5, 63.5$ (CH₂), 70.3 (CH), 84.7, 85.8 (C \equiv C), 120.8, 123.9, 126.9, 127.8, 128.9, 129.0, 129.0, 129.2, 129.3, 130.1, 131.7, 132.6, 133.5, 133.5 (CH_{Ar}), 121.9, 126.3, 133.6, 134.2, 134.6, 143.0 (C_{Ar}), 172.0, 178.9, 180.7 (C). IR (ATR, cm⁻¹): $\tilde{\nu} = 3059$ (br, w), 2953 (br, w), 2870 (br, w), 1732 (w), 1672 (s), 1634 (s), 1439 (m), 1364 (m), 1336 (s), 1254 (s), 1164 (m), 1088 (m), 750 (s), 701 (s), 687 (m), 570 (w). MS (EI, 70 eV): m/z (%) = 645 (M⁺, 52), 601 (31), 510 (11), 453 (12), 414 (8), 264 (16), 217 (98), 160 (100), 91 (89), 65 (13), 44 (16). HRMS (ESI): calcd for C₃₆H₃₀³⁵Cl₁N₃NiO₃ ([M]) 645.13237, found 645.13405, calcd for C₃₆H₃₀³⁷Cl₁N₃NiO₃ ([M]) 647.12942, found 647.13164.

(S)-2-Amino-5-[4-(trifluoromethyl)benzene]pent-4-ynoic acid-Ni-(S)-BPB (2i): Reaction of **1** (134 mg, 0.25 mmol), Pd(PPh₃)₄ (14.4 mg, 0.013 mmol), CuI (4.8 mg, 0.025 mmol) and 1-bromo-4-(trifluoromethyl)benzene (41.4 μ l, 0.3 mmol) in HNiPr₂ (1 ml) and 1,4-dioxane (1

ml) for 3 h gave **2i** as a red solid (125 mg, 74%); mp 142-143 °C. ^1H NMR (250 MHz, CDCl_3): δ = 1.56–1.71 (m, 1H, $\text{CH}_{2,\text{Pro}}$), 1.94–2.15 (m, 3H, $\text{CH}_{2,\text{Pro}}$), 2.54 (dd, 3J = 6.8 Hz, 2J = 17.2 Hz, 1H, $\text{CH}_{2,\text{Gly}}$), 2.92 (dd, 3J = 2.9 Hz, 2J = 17.2 Hz, 1H, $\text{CH}_{2,\text{Gly}}$), 3.05–3.24 (m, 1H, $\text{CH}_{2,\text{Pro}}$), 3.36 (dd, 3J = 7.5 Hz, 3J = 9.3 Hz, 1H, CH_{Pro}), 3.54 (d, 2J = 12.6 Hz, 1H, CH_2Ph), 3.50–3.60 (m, 1H, $\text{CH}_{2,\text{Pro}}$), 4.07–4.14 (m, 1H, CH_{Gly}), 4.40 (d, 2J = 12.6 Hz, 1H, CH_2Ph), 6.68 (d, J = 4.2 Hz, 2H, C_6H_4), 7.06 (d, J = 6.7 Hz, 1H, Ar), 7.13–7.39 (m, 5H, Ar), 7.44–7.61 (m, 7H, Ar), 8.05 (d, J = 7.2 Hz, 2H, C_6H_5), 8.22 (d, J = 8.7 Hz, 1H, C_6H_4). ^{13}C NMR(62.9 MHz, CDCl_3): δ = 23.6, 24.8, 30.5, 57.5, 63.6 (CH_2), 68.0, 70.3 (CH), 84.5, 87.5 ($\text{C} \equiv \text{C}$), 120.9, 124.0 (CH_{Ar}), 125.6 (q, 3J = 3.8 Hz, $\text{CH}_{\text{CF}_3\text{Ph}}$), 126.3 (C_{Ar}), 127.0 (CH_{Ar}), 127.2 (bs, $\text{C}_{\text{CF}_3\text{Ph}}$), 127.9, 129.1, 129.2, 129.3, 129.4, 130.2 (CH_{Ar}), 130.4 (q, 2J = 32.0 Hz, $\text{CH}_{\text{CF}_3\text{Ph}}$), 131.8, 132.6, 132.8, 133.7 (CH_{Ar}), 133.5, 134.3, 143.1 (C_{Ar}), 172.2, 178.9, 180.7 (C). ^{19}F NMR (282.4 MHz, CDCl_3): δ = -62.8. IR (ATR, cm^{-1}): $\tilde{\nu}$ = 3059 (br, w), 2964 (br, w), 2870 (br, w), 1733 (w), 1673 (m), 1635 (s), 1321 (s), 1255 (s), 1163 (s), 1121 (s), 1104 (s), 1065 (s), 1015(m), 843 (m), 750 (s), 701 (s), 686 (m), 599 (m), 569 (m). MS (EI, 70 eV): m/z (%) = 679 (M^+ , 58), 635 (33), 544 (9), 264 (7), 217 (56), 160 (100), 91 (95). HRMS (EI): calcd for $\text{C}_{37}\text{H}_{30}\text{F}_3\text{N}_3\text{NiO}_3$ ($[\text{M}]^+$) 679.15873, found 679.16111. Anal.Calcd for $\text{C}_{37}\text{H}_{30}\text{F}_3\text{N}_3\text{NiO}_3$ (680.4): C, 65.32; H, 4.44; N, 6.18. Found: C, 65.12; H, 4.33; N, 5.84.

(S)-2-Amino-5-[3-methoxybenzene]pent-4-ynoic acid-Ni-(S)-BPB (2j): Reaction of **1** (134 mg, 0.25 mmol), $\text{Pd}(\text{PPh}_3)_4$ (14.4 mg, 0.013 mmol), CuI (4.8 mg, 0.025 mmol) and 1-bromo-4-methoxybenzene (37.7 μl , 0.3 mmol) in HNiPr_2 (1 ml) and 1,4-dioxane (1 ml) for 3 h gave **2j** as a red solid (96 mg, 60%); mp 200-201 °C. ^1H NMR (250 MHz, CDCl_3): δ = 1.53–1.69 (m, 1H, $\text{CH}_{2,\text{Pro}}$), 1.97–2.17 (m, 3H, $\text{CH}_{2,\text{Pro}}$), 2.50 (dd, 3J = 6.7 Hz, 2J = 17.2 Hz, 1H, $\text{CH}_{2,\text{Gly}}$), 2.91 (dd, 3J = 2.9 Hz, 2J = 17.2 Hz, 1H, $\text{CH}_{2,\text{Gly}}$), 3.04–3.22 (m, 1H, $\text{CH}_{2,\text{Pro}}$), 3.32 (dd, 3J = 7.5 Hz, 3J = 9.4 Hz, 1H, CH_{Pro}), 3.54 (d, 2J = 12.6 Hz, 1H, CH_2Ph), 3.58–3.69 (m, 1H, $\text{CH}_{2,\text{Pro}}$), 3.74 (s, 3H, CH_3), 4.05–4.15 (m, 1H, CH_{Gly}), 4.40 (d, 2J = 12.6 Hz, 1H, CH_2Ph), 6.67 (d, J = 3.7 Hz, 2H, C_6H_4), 6.83–6.90 (m, 1H, Ar), 6.97–7.01 (m, 1H, Ar), 7.06–7.56 (m, 11H, Ar), 8.06 (d, J = 7.1 Hz, 2H, C_6H_5), 8.24 (d, J = 8.7 Hz, 1H, C_6H_4). ^{13}C NMR(62.9 MHz, CDCl_3): δ = 23.7, 24.8, 30.6, 57.8, 63.6 (CH_2), 55.5 (CH_3), 70.4 (CH), 84.5, 85.9 ($\text{C} \equiv \text{C}$), 115.7, 116.5, 120.8, 124.0, 124.9, 126.3, 127.9, 129.0, 129.3, 129.7, 130.1, 131.8, 132.6, 133.6 (CH_{Ar}), 124.4, 127.0, 133.6, 134.4, 143.2, 159.6 (C_{Ar}), 172.0, 179.0, 180.8 (C).IR (ATR, cm^{-1}): $\tilde{\nu}$ = 3058 (br, w), 2955 (br, w), 2869 (br, w), 1732 (w), 1672 (s), 1634 (s), 1583 (m), 1438 (m), 1364 (m), 1335 (s), 1314 (m), 1286 (m), 1254 (s), 1163 (s), 750 (s), 701 (s), 687 (s), 540 (m). MS (EI, 70 eV): m/z (%) = 641 (M^+ , 62), 597 (21), 506 (8), 410 (9), 264 (8),

217 (54), 160 (100), 91 (85), 44 (6). HRMS (EI): calcd for $C_{37}H_{33}N_3NiO_4$ ($[M]$) 641.18191, found 641.18378.

(S)-2-Amino-5-[4-nitrobenzene]pent-4-ynoic acid-Ni-(S)-BPB (2k): Reaction of **1** (804 mg, 1.5 mmol), $Pd(PPh_3)_4$ (86.0 mg, 0.075 mmol), CuI (28.6 mg, 0.150 mmol) and 1-bromo-4-nitrobenzene (363.6 mg, 1.8 mmol) in $HNiPr_2$ (6 ml) and 1,4-dioxane (6 ml) for 3 h gave **2k** as a red solid (66 mg, 7%); mp 145–146 °C. 1H NMR (300 MHz, $CDCl_3$): δ = 1.58–1.73 (m, 1H, $CH_{2,Pro}$), 1.94–2.19 (m, 3H, $CH_{2,Pro}$), 2.60 (dd, $^3J = 7.0$ Hz, $^2J = 17.3$ Hz, 1H, $CH_{2,Gly}$), 2.95 (dd, $^3J = 3.0$ Hz, $^2J = 17.3$ Hz, 1H, $CH_{2,Gly}$), 3.14–3.28 (m, 1H, $CH_{2,Pro}$), 3.34–3.43 (m, 1H, CH_{Pro}), 3.50–3.60 (m, 2H, $CH_2Ph/CH_{2,Pro}$), 4.05–4.16 (m, 1H, CH_{Gly}), 4.39 (d, $J = 12.6$ Hz, 1H, CH_2Ph), 6.68 (d, $J = 3.2$ Hz, 2H, C_6H_4), 7.05 (d, $J = 7.2$ Hz, 1H, Ar), 7.15–7.63 (m, 11H, Ar), 8.05 (d, $J = 7.0$ Hz, 2H, C_6H_5), 8.17–8.20 (m, 2H, Ar). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 23.7, 25.0, 30.6, 57.4, 63.6 (CH_2), 70.3 (CH), 84.0, 90.6 ($C \equiv C$), 121.0, 123.9, 124.0, 127.0, 127.8, 129.1, 129.2, 129.4, 129.5, 130.3, 131.8, 132.9, 133.0, 133.5 (CH_{Ar}), 126.2, 130.2, 133.7, 134.2, 143.0, 147.4 (C_{Ar}), 172.4, 178.7, 180.8 (C). IR (ATR, cm^{-1}): $\tilde{\nu} = 3060$ (br, w), 2963 (br, w), 2868 (br, w), 1673 (m), 1635 (s), 1590 (m), 1515 (m), 1438 (m), 1338 (s), 1309 (m), 1164 (m), 852 (m), 749 (s), 700 (s), 689 (m), 541 (m). MS (EI, 70 eV): m/z (%) = 656 (M^+ , 20), 626 (11), 612 (18), 439 (9), 342 (17), 217 (40), 180 (7), 160 (100), 91 (98), 44 (18). HRMS (EI): calcd for $C_{36}H_{30}N_4NiO_5$ ($[M]$) 656.15642, found 656.15590.

5,5'-[1,4-Benzene]bis((S)-2-aminopent-4-ynoic acid-Ni-(S)-BPB) (2l): Reaction of **1** (1600 mg, 3.00 mmol), $Pd(PPh_3)_4$ (173.3 mg, 0.15 mmol), CuI (57.2 mg, 0.30 mmol) and 1,4-dibromobenzene (353.9 mg, 1.50 mmol) in $HNiPr_2$ (12 ml) and 1,4-dioxane (12 ml) for 4.5 h gave **2l** as a red solid (785 mg, 46%); mp 205–206 °C. 1H NMR (250 MHz, $CDCl_3$): δ = 1.56–1.75 (m, 2H, $CH_{2,Pro}$), 1.92–2.18 (m, 6H, $CH_{2,Pro}$), 2.45–2.64 (m, 2H, $CH_{2,Gly}$), 2.90 (d, $^2J = 16.9$ Hz, 2H, $CH_{2,Gly}$), 3.18–3.38 (m, 4H, $CH_{2,Pro}/CH_{Pro}$), 3.57 (d, $^2J = 12.7$ Hz, 2H, CH_2Ph), 3.47–3.62 (m, 2H, $CH_{2,Pro}$), 4.02–4.14 (m, 2H, CH_{Gly}), 4.40 (d, $J = 12.7$ Hz, 2H, CH_2Ph), 6.67 (d, $J = 3.2$ Hz, 4H, C_6H_4), 7.04–7.60 (m, 22H, Ar), 8.04 (d, $J = 7.1$ Hz, 4H, C_6H_5), 8.21 (d, $J = 8.1$ Hz, 2H, C_6H_4). ^{13}C NMR (62.9 MHz, $CDCl_3$): δ = 23.7, 24.9, 30.7, 57.5, 63.5 (CH_2), 68.5, 70.3 (CH), 85.3, 86.7 ($C \equiv C$), 120.8, 123.9, 126.9, 127.9, 129.0, 129.0, 129.3, 130.2, 131.8, 132.3, 132.7, 133.7, (CH_{Ar}), 123.4, 126.4, 133.5, 134.3, 143.1 (C_{Ar}), 172.0, 179.0, 180.7 (C). IR (ATR, cm^{-1}): $\tilde{\nu} = 3057$ (br, w), 2960 (br, w), 2867 (br, w), 1673 (m), 1634 (s), 1585 (m), 1438 (m), 1362 (m), 1334 (s), 1312 (m), 1252 (s), 1163 (m), 749 (s), 701 (s), 687 (m), 547 (m). HRMS (ESI): calcd for $C_{66}H_{56}N_6Ni_2O_6$ ($[M+H]^+$) 1145.30411, found 1145.30343, calcd for $^{13}C_1^{12}C_{65}H_{56}N_6Ni_2O_6$ ($[M+H]^+$) 1146.30730, found 1146.30674,

calcd for $^{13}\text{C}_2^{12}\text{C}_{64}\text{H}_{56}\text{N}_6\text{Ni}_2\text{O}_6$ ($[\text{M}+\text{H}]^+$) 1147.30248, found 1147.30206, calcd for $\text{C}_{66}\text{H}_{56}\text{N}_6\text{NaNi}_2\text{O}_6$ ($[\text{M}+\text{Na}]^+$) 1167.28605, found 1167.28519, calcd for $^{13}\text{C}_1^{12}\text{C}_{65}\text{H}_{56}\text{N}_6\text{NaNi}_2\text{O}_6$ ($[\text{M}+\text{Na}]^+$) 1168.28925, found 1168.28788, calcd for $^{13}\text{C}_2^{12}\text{C}_{64}\text{H}_{56}\text{N}_6\text{NaNi}_2\text{O}_6$ ($[\text{M}+\text{Na}]^+$) 1169.28443, found 1169.28372.

General procedure for 3a-j and 3l: Starting with **2a-j** and **2l** (0.4 – 1.1 mmol) respectively: the Ni-complex was dissolved in 10 ml MeOH and afterwards 5 ml H₂O as well as 1.5 ml HCl (12M) were added. The stirred mixture was heated to 60 °C for 20 min (the color changed from red to green). After cooling to room temperature the mixture was diluted with water and extracted with DCM four times to remove (S)-BPB. The H₂O layer was treated with 5% NH₄OH until pH 1. Afterwards the amino acid was separated from the mixture by cation exchange column (dowex 50WX8 H⁺) to obtain **3a-j** and **3l** as a white solid.

(S)-2-Amino-5-[phenyl]pent-4-ynoic acid (3a): Reaction of **2a** (580 mg, 0.949 mmol) gave **3a** as a white solid (156 mg, 87%). Mp 238-239 °C; $[\alpha]_{\text{D}}^{27}$ -10.4 (c 0.20, 1M HCl). ¹H NMR (300 MHz, CD₃OD, pH = 1): δ = 3.12 (dd, ²J = 17.9 Hz, ³J = 5.0 Hz, 1H, CH₂), 3.22 (d, ²J = 17.9 Hz, ³J = 5.4 Hz, 1H, CH₂), 4.26 (dd, ³J = 5.0 Hz, ³J = 5.4 Hz, 1H, CH), 7.31–7.37 (m, 3H, Ph), 7.43–7.48 (m, 2H, Ph). ¹³C NMR(75 MHz, CD₃OD, pH = 1): δ = 22.4 (CH₂), 52.9 (CH), 82.4, 85.8 (C ≡ C), 124.0 (C_{Ph}), 129.5, 129.8, 133.0 (CH_{Ph}), 170.5 (COOH). IR (ATR, cm⁻¹): $\tilde{\nu}$ = 2914 (br, w), 2079 (br, w), 1577 (s), 1504 (s), 1489 (s), 1424 (s), 1403 (s), 1318 (s), 1136 (m), 1112 (m), 1069 (m), 844 (m), 752 (s), 691 (s), 527 (m), 500 (m). MS (EI, 70 eV): *m/z* (%) = 190 (M⁺, 10), 162 (40), 133 (58), 109 (9), 47 (100), 44 (8). HRMS (ESI): calcd for C₁₁H₁₂NO₂ ($[\text{M}+\text{H}]^+$) 190.08626, found 190.08617.

(S)-2-Amino-5-[4-tolyl]pent-4-ynoic acid (3b): Reaction of **2b** (510 mg, 0.816 mmol) gave **3b** as a white solid (147 mg, 89%). Mp 239-240 °C; $[\alpha]_{\text{D}}^{27}$ -16.2 (c 0.20, 1M HCl). ¹H NMR (300 MHz, CD₃OD, pH = 1): δ = 2.33 (s, 3H, CH₃), 3.15 (dd, ²J = 17.8 Hz, ³J = 5.0 Hz, 1H, CH₂), 3.16 (dd, ²J = 17.8 Hz, ³J = 5.5 Hz, 1H, CH₂), 4.26 (dd, ³J = 5.0 Hz, ³J = 5.5 Hz, CH), 7.14 (d, ³J = 7.9 Hz, 2H, Ar), 7.33 (d, ³J = 7.9 Hz, 2H, Ar). ¹³C NMR(75 MHz, CD₃OD, pH = 1): δ = 21.6 (CH₃), 22.4 (CH₂), 52.9 (CH), 81.7, 86.1 (C ≡ C), 121.0, 140.1 (C_{Ar}), 130.2, 132.9 (CH_{Ar}), 170.5 (COOH). IR (ATR, cm⁻¹): $\tilde{\nu}$ = 3128 (br, m), 3026 (br, m), 2865 (br, m), 2081 (br, w), 1759 (br, w), 1605 (m), 1576 (m), 1508 (s), 1489 (m), 1423 (s), 1401 (s), 1367

(m), 1321 (s), 1134 (m), 1107 (m), 816 (s), 528 (s). MS (EI, 70 eV): m/z (%) = 203 (M^+ , 7), 186 (5), 171 (3), 159 (100), 143 (11), 129 (41), 115 (16), 105 (48), 91 (11), 74 (11), 44 (29). HRMS (ESI): calcd for $C_{12}H_{12}NO_2$ ($[M-H]^-$) 202.08735, found 202.0696.

(S)-2-Amino-5-[3-tolyl]pent-4-ynoic acid (3c): Reaction of **2c** (521 mg, 0.833 mmol) gave **3c** as a white solid (144 mg, 87%). Mp 228-229 °C; $[\alpha]_D^{24} -11.3$ (c 0.20, 1M HCl). 1H NMR (300 MHz, D_2O , pH = 1): δ = 2.09 (s, 3H, CH_3), 2.93 (dd, $^2J = 18.0$ Hz, $^3J = 4.9$ Hz, 1H, CH_2), 3.04 (dd, $^2J = 18.0$ Hz, $^3J = 5.8$ Hz, 1H, CH_2), 4.19 (dd, $^3J = 4.9$ Hz, $^3J = 5.8$ Hz, 1H, CH), 7.05–7.10 (m, 3H, Ar), 7.13 (bs, 1H, Ar). ^{13}C NMR (62.9 MHz, D_2O , pH = 1): δ = 20.1 (CH_3), 20.7 (CH_2), 51.4 (CH), 81.2, 84.8 ($C \equiv C$), 121.5, 138.7 (C_{Ar}), 128.4, 128.6, 129.7, 134.0 (CH_{Ar}), 170.2 (COOH). IR (ATR, cm^{-1}): $\tilde{\nu}$ = 3174 (br, w), 2962 (br, m), 1599 (s), 1581 (s), 1505 (s), 1486 (m), 1425 (s), 1402 (s), 1351 (m), 1319 (s), 1113 (m), 798 (m), 784 (s), 693 (s), 530 (m). MS (EI, 70 eV): m/z (%) = 204 (M^+ , 2), 186 (76), 158 (28), 144 (10), 129 (100), 115 (22), 91 (7), 75 (41), 36 (26). HRMS (ESI): calcd for $C_{12}H_{14}NO_2$ ($[M+H]^+$) 204.10191, found 204.10209, calcd for $C_{12}H_{13}NaNO_2$ ($[M+Na]^+$) 226.08385, found 226.0839.

(S)-2-Amino-5-[2-tolyl]pent-4-ynoic acid (3d): Reaction of **2d** (379 mg, 0.606 mmol) gave **3d** as a white solid (88 mg, 72%). Mp 197-198 °C; $[\alpha]_D^{25} -10.4$ (c 0.23, 1M HCl). 1H NMR (250 MHz, D_2O , pH = 1): δ = 2.37 (s, 3H, CH_3), 3.16 (dd, $^2J = 17.9$ Hz, $^3J = 4.8$ Hz, 1H, CH_2), 3.31 (dd, $^2J = 17.9$ Hz, $^3J = 5.5$ Hz, 1H, CH_2), 4.40 (dd, $^3J = 4.8$ Hz, $^3J = 5.5$ Hz, 1H, CH), 7.12–7.47 (m, 4H, Ar). ^{13}C NMR (62.9 MHz, D_2O , pH = 1): δ = 19.7 (CH_3), 21.1 (CH_2), 51.7 (CH), 83.6, 85.5 ($C \equiv C$), 121.4, 140.8 (C_{Ar}), 125.7, 129.1, 129.5, 132.1 (CH_{Ar}), 170.4 (COOH). IR (ATR, cm^{-1}): $\tilde{\nu}$ = 3117 (br, w), 3043 (br, m), 2956 (br, m), 1606 (s), 1419 (m), 1403 (s), 1331 (m), 752 (s), 549 (m), 531 (s). MS (EI, 70 eV): m/z (%) = 203 (M^+ , 3), 186 (3), 171 (3), 158 (27), 143 (8), 128 (44), 115 (20), 91 (6), 74 (100), 44 (8). HRMS (ESI): calcd for $C_{12}H_{12}NO_2$ ($[M-H]^-$) 202.08735, found 202.08696.

(S)-2-Amino-5-[naphth-1-yl]pent-4-ynoic acid (3e): Reaction of **2e** (604 mg, 0.914 mmol) gave **3e** as a white solid (129 mg, 54%). Mp 223-224 °C; $[\alpha]_D^{26} -11.4$ (c 0.19, 1M HCl). 1H

NMR (300 MHz, CD₃OD, pH = 1): δ = 3.30 (dd, 2J = 17.8 Hz, 3J = 5.0 Hz, 1H, CH₂), 3.40 (d, 2J = 17.8 Hz, 3J = 5.4 Hz, 1H, CH₂), 4.37 (dd, 3J = 5.0 Hz, 3J = 5.4 Hz, 1H, CH), 7.44 (dd, 3J = 7.1 Hz, 3J = 8.0 Hz, 1H, Ar), 7.51–7.63 (m, 2H, Ar), 7.70 (dd, 4J = 0.8 Hz, 3J = 7.1 Hz, 1H, Ar), 7.88 (d, 3J = 8.4 Hz, 2H, Ar), 8.30 (d, 3J = 8.0 Hz, 1H, Ar). ¹³C NMR(75 MHz, CD₃OD, pH = 1): δ = 22.7 (CH₂), 53.0 (CH), 84.0, 87.5 (C \equiv C), 121.5 (C_{Ar}), 126.4, 127.2, 127.7, 128.1, 129.5, 130.2, 132.1 (CH_{Ar}), 134.7, 134.8 (C_{Ar}), 170.6 (COOH). IR (ATR, cm⁻¹): $\tilde{\nu}$ = 3120 (br, m), 3041 (br, m), 2832 (br, m), 1756 (br, w), 1587 (m), 1504 (m), 1396 (s), 1322 (m), 1097 (br, m), 1063 (br, m), 1016 (m), 799 (s), 770 (s), 615 (m), 564 (m). MS (EI, 70 eV): m/z (%) = 239 (M⁺, 54), 222 (13), 167 (82), 165 (100), 152 (10), 139 (8), 128 (9), 74 (26). HRMS (ESI): calcd for C₁₅H₁₂NO₂ ([M-H]⁻) 238.08735, found 238.0869.

(S)-2-Amino-5-[thiophen-2-yl]pent-4-ynoic acid (3f): Reaction of **2f** (649 mg, 1.050 mmol) gave **3f** as a white solid (180 mg, 88%). Mp 230-231 °C; $[\alpha]_D^{28}$ -9.3 (*c* 0.20, 1M HCl). ¹H NMR (300 MHz, CD₃OD, pH = 1): δ = 3.15 (d, 2J = 17.8 Hz, 3J = 5.0 Hz, 1H, CH₂), 3.32 (d, 2J = 17.8 Hz, 3J = 5.6 Hz, 1H, CH₂), 4.26 (dd, 3J = 5.0 Hz, 3J = 5.6 Hz, 1H, CH), 7.00 (dd, 3J = 3.6 Hz, 3J = 5.1 Hz, 1H, Ar), 7.25 (dd, 4J = 1.1 Hz, 3J = 3.6 Hz, 1H, Ar), 7.25 (dd, 4J = 1.1 Hz, 3J = 5.1 Hz, 1H, Ar). ¹³C NMR(62.9 MHz, CD₃OD, pH = 1): δ = 22.6 (CH₂), 52.7 (CH), 79.2, 86.5 (C \equiv C), 133.0 (C_{Ar}), 128.2, 128.8, 133.8 (CH_{Ar}), 170.3 (COOH). IR (ATR, cm⁻¹): $\tilde{\nu}$ = 3012 (br, m), 2600 (br, w), 2084 (br, w), 1576 (m), 1504 (s), 1420 (s), 1405 (s), 1316 (m), 828 (m), 695 (s). MS (EI, 70 eV): m/z (%) = 195 (M⁺, 0), 152 (84), 123 (100), 109 (21), 96 (18), 45 (9). HRMS (ESI): calcd for C₉H₈NO₂S ([M-H]⁻) 194.02812, found 194.02767.

(S)-2-Amino-5-[4-fluorobenzene]pent-4-ynoic acid (3g): Reaction of **2g** (529 mg, 0.841 mmol) gave **3g** as a white solid (156 mg, 90%). Mp 228-229 °C; $[\alpha]_D^{25}$ -9.8 (*c* 0.20, 1M HCl); ee > 99% (HPLC). ¹H NMR (250 MHz, D₂O, pH = 1): δ = 3.08 (dd, 2J = 18.0 Hz, 3J = 5.4 Hz, 1H, CH₂), 3.17 (dd, 2J = 18.0 Hz, 3J = 5.8 Hz, 1H, CH₂), 4.33 (dd, 3J = 5.4 Hz, 3J = 5.8 Hz, 1H, CH), 7.01–7.13 (m, 2H, Ar), 7.41–7.60 (m, 2H, Ar). ¹³C NMR(62.9 MHz, D₂O, pH = 1): δ = 22.4 (CH₂), 53.1 (CH), 82.7, 85.5 (C \equiv C), 117.2 (d, 2J = 22.3 Hz, CH_{Ar}), 119.4 (d, 4J = 3.4 Hz, C_{Ar}), 135.4 (d, 3J = 8.7 Hz, CH_{Ar}), 164.1 (d, 1J = 247.3 Hz, CF), 171.9 (COOH). ¹⁹F NMR (282.4 MHz, CD₃OD, pH = 1): δ = -111.0. IR (ATR, cm⁻¹): $\tilde{\nu}$ = 3161 (br, m), 2991 (br, m), 2914 (br, m), 1601 (s), 1505 (s), 1479 (s), 1426 (s), 1405 (s), 1356 (m), 1323 (m), 1219 (s), 1148 (m), 1110 (m), 1094 (m), 833 (s), 811 (m), 628 (m), 526 (s). MS

(EI, 70 eV): m/z (%) = 207 (M^+ , 2), 190 (11), 162 (49), 133 (61), 115 (18), 91 (7), 74 (100), 44 (37). HRMS (ESI): calcd for $C_{11}H_{19}FNO_2$ ($[M-H]^+$) 206.06228, found 206.06284.

(S)-2-Amino-5-[4-chlorobenzene]pent-4-ynoic acid (3h): Reaction of **2h** (612 mg, 0.949 mmol) gave **3h** as a white solid (179 mg, 84%). Mp 225-226 °C; $[\alpha]_D^{27}$ -21.0 (c 0.20, 1M HCl). 1H NMR (300 MHz, CD_3OD , pH = 1): δ = 3.13 (dd, 2J = 18.0 Hz, 3J = 4.9 Hz, 1H, CH_2), 3.19 (dd, 2J = 18.0 Hz, 3J = 5.3 Hz, 1H, CH_2), 4.27 (dd, 3J = 4.9 Hz, 3J = 5.3 Hz, 1H, CH), 7.34 (d, 3J = 8.6 Hz, 2H, Ar), 7.44 (d, 3J = 8.6 Hz, 2H, Ar). ^{13}C NMR(75 MHz, CD_3OD , pH = 1): δ = 22.4 (CH_2), 52.9 (CH), 83.9, 84.8 ($C \equiv C$), 122.8, 135.7 (C_{Ar}), 129.8, 134.5 (CH_{Ar}), 170.4 (COOH). IR (ATR, cm^{-1}): $\tilde{\nu}$ = 3130 (br, m), 3039 (br, m), 1771 (br, w), 1604 (m), 1591 (m), 1508 (m), 1488 (s), 1425 (m), 1401 (s), 1367 (m), 1356 (m), 1323 (s), 1149 (m), 1110 (m), 1089 (s), 1069 (m), 1016 (m), 823 (s), 637 (m). MS (EI, 70 eV): m/z (%) = 223 (M^+ , 2), 206 (5), 178 (47), 149 (19), 125 (11), 115 (35), 59 (7), 74 (100), 63 (6), 44 (19). HRMS (ESI): calcd for $C_{11}H_9^{35}ClNO_2$ ($[M-H]^+$) 222.03273, found 222.03226, calcd for $C_{11}H_9^{37}ClNO_2$ ($[M-H]^+$) 224.03007, found 224.02953.

(S)-2-Amino-5-[4-(trifluoromethyl)benzene]pent-4-ynoic acid (3i): Reaction of **2i** (748 mg, 1.100 mmol) gave **3i** as a white solid (261 mg, 92%). Mp 220-221 °C; $[\alpha]_D^{27}$ -10.7 (c 0.25, 1M HCl). 1H NMR (300 MHz, CD_3OD , pH = 1): δ = 3.22 (d, 3J = 5.0 Hz, 1H, CH_2), 3.22 (d, 3J = 5.4 Hz, 1H, CH_2), 4.32 (dd, 3J = 5.0 Hz, 3J = 5.4 Hz, 1H, CH), 7.63–7.67 (m, 4H, Ar). ^{13}C NMR(75 MHz, CD_3OD , pH = 1): δ = 22.4 (CH_2), 52.7 (CH), 84.5, 85.7 ($C \equiv C$), 125.5 (q, 1J = 271 Hz, CF_3), 126.5 (q, 3J = 3.8 Hz, CH_{Ar}), 128.2 (C_{Ar}), 131.3 (q, 2J = 32.2 Hz, (C_{Ar}), 133.6 (CH_{Ar}), 170.3 (COOH). ^{19}F NMR (282.4 MHz, CD_3OD , pH = 1): δ = -64.3. IR (ATR, cm^{-1}): $\tilde{\nu}$ = 3132 (br, m), 3040 (br, m), 2619 (br, w), 2088 (br, w), 1767 (br, w), 1607 (m), 1575 (m), 1507 (m), 1480 (m), 1425 (m), 1404 (s), 1321 (s), 1291 (m), 1168 (s), 1126 (s), 1104 (s), 1068 (s), 1019 (m), 842 (s), 596 (m). MS (EI, 70 eV): m/z (%) = 257 (M^+ , 1), 240 (12), 212 (47), 183 (31), 164 (16), 143 (21), 115 (24), 74 (100). HRMS (ESI): calcd for $C_{12}H_9F_3NO_2$ ($[M-H]^+$) 256.05909, found 256.05876.

(S)-2-Amino-5-[3-methoxybenzene]pent-4-ynoic acid (3j): Reaction of **2j** (280 mg, 0.436 mmol) gave **3j** as a white solid (94 mg, 99%). Mp 209-210 °C; $[\alpha]_D^{26} -10.2$ (*c* 0.20, 1M HCl); ee > 99% (HPLC). ¹H NMR (250 MHz, D₂O, pH = 1): δ = 3.15 (dd, ²*J* = 18.0 Hz, ³*J* = 5.2 Hz, 1H, CH₂), 3.22 (dd, ²*J* = 18.0 Hz, ³*J* = 6.0 Hz, 1H, CH₂), 3.79 (s, 3H, CH₃) 4.25 (dd, ³*J* = 5.2 Hz, ³*J* = 6.0 Hz, 1H, CH), 6.98–7.17 (m, 3H, Ar), 7.28–7.45 (m, 1H, Ar). ¹³C NMR (62.9 MHz, D₂O, pH = 1): δ = 22.4 (CH₂), 53.1 (CH), 57.0 (CH₃), 82.3, 86.0 (C ≡ C), 116.7, 118.5, 126.2, 131.5 (CH_{Ar}), 124.5, 160.2 (C_{Ar}), 172.0 (COOH). IR (ATR, cm⁻¹): $\tilde{\nu}$ = 3126 (br, m), 3009 (br, m), 2835 (br, m), 2087 (br, w), 1766 (br, w), 1583 (s), 1507 (s), 1489 (s), 1469 (m), 1404 (s), 1351 (m), 1317 (s), 1288 (s), 1267 (m), 1208 (s), 1179 (m), 1108 (br, m), 1037 (s), 860 (s), 785 (s), 689 (m), 615 (m), 534 (s). MS (EI, 70 eV): *m/z* (%) = 219 (M⁺, 26), 202 (27), 174 (100), 159 (11), 145 (36), 115 (21), 91 (9), 74 (76), 44 (20). HRMS (ESI): calcd for C₁₂H₁₂NO₃ ([M-H]⁻) 218.08227, found 218.08186.

5,5'-[1,4-benzene]bis((S)-2-aminopent-4-ynoic acid) (3l): Reaction of **2l** (602 mg, 0.525 mmol) gave **3l** as a white solid (133 mg, 85%). Mp 260 °C (decomposition); $[\alpha]_D^{27} -31.1$ (*c* 0.18, 1M HCl). ¹H NMR (300 MHz, CD₃OD, pH = 1): δ = 3.14 (dd, ²*J* = 17.9 Hz, ³*J* = 5.0 Hz, 2H, CH₂), 3.21 (dd, ²*J* = 17.9 Hz, ³*J* = 5.4 Hz, 2H, CH₂), 4.28 (dd, ³*J* = 5.0 Hz, ³*J* = 5.4 Hz, 2H, CH), 7.43 (s, 4H, Ar). ¹³C NMR (62.9 MHz, CD₃OD, pH = 1): δ = 22.4 (CH₂), 52.8 (CH), 84.7, 85.4 (C ≡ C), 124.3 (C_{Ar}), 133.0 (CH_{Ar}), 170.4 (COOH). IR (ATR, cm⁻¹): $\tilde{\nu}$ = 3120 (br, w), 2993 (br, m), 2660 (br, w), 1607 (br, s), 1541 (s), 1502 (m), 1424 (m), 1403 (s), 1370 (s), 1332 (s), 1070 (m), 842 (s), 610 (m), 560 (m), 531 (s). HRMS (ESI): calcd for C₁₆H₁₅N₂O₄ ([M-H]⁻) 299.10373, found 299.10457.

OSIRIS Drug Properties and Toxicity Profile

The novel synthesized series of compounds were evaluated for their potential to qualify as drug candidate by using OSIRIS property explorer²⁶ based on Lipinski's rule of five.²⁵ Additionally, the OSIRIS property explorer was also used to determine the toxicity of the synthesized series. Toxicity profiles such as tumorigenic, mutagenic, irritability and reproductive toxicity were calculated using the same software.

Enzyme part

Materials

Coenzyme nicotinamide adenine dinucleotide phosphate (NADPH), (D, L-glyceraldehyde), quercetin were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). All the solvents of highest purity used were bought from Merck, Fluka while valporate acid was purchased from local pharmaceutical store.

Extraction and activity of ALR1 and ALR2

Isolation and purification of aldehyde reductase enzyme (ALR1): ALR1 was purified from bovine kidney using extraction method previously described.³¹ Briefly, bovine kidneys were collected from a local abattoir were diced and homogenised in three volumes of 10 mM sodium phosphate buffer (pH 7.2) containing 0.25 M sucrose, 2.0 mM EDTA and 2.5 mM b-mercaptoethanol. The homogenate was centrifuged at 10000 g and -4 °C for 30 mins, obtained supernatant was fractionated by the addition of 40-60 % ammonium sulphate. The ammonium sulphate concentration was further increased to 75 % to have ALR1 been precipitated. And this was performed at the operation condition of -4 °C. The purified enzyme in pellet form was suspended in 10 mM sodium phosphate buffer (pH 7.2) with 2.0 mM of EDTA and 2.5 mM b-mercaptoethanol in it and dialyzed over the night with the same buffer. The volume dialyzed enzyme was noted and stored at -80 °C and was subsequently used for enzymatic assays.³⁶

Isolation and purification of aldose reductase enzyme (ALR2): Isolation and purification of ALR2 enzyme was carried out using calf lenses according to the modified method previously described.³⁷ The lenses were quickly removed from the calf eyes into a glass homogenizer surrounded with ice and homogenized with 10 ml of 10 mM phosphate buffer of pH 7.0 that contains the mixture of 1 mM b-mercaptoethanol and 1 mM EDTA, centrifuged at 18000 g at -4 °C for 20 min. Ammonium sulphate was slowly added to the stirred supernatant fraction that yielded a 35 % saturated solution. After the completion of the stirring for 3 h, the solution was further centrifuged at 18000 g for the period of 20 mins and precipitated. The precipitates were dissolved in buffer of phosphate (pH 7.0) and dialysed for two days using the same buffer. The purified ALR2 was store at -80 °C until was used in the enzyme assays.

ALR1 and ALR2 enzyme assays

ALR1 enzyme assay: The enzymatic activities of ALR1 and ALR2 were determined by assayed spectrophotometrically using a microplate reader (Bio-Tek ELx 800™, Instruments Inc., Winooski, VT, USA) which measures the decrease in the absorption of NADPH that follows by the oxidation of NADPH catalyzed by both enzymes. The activity of ALR1 was measured according to the reported procedure³⁸, assayed spectrophotometrically by

determining NADPH consumption at 340 nm. The reaction mixture consists of 20 μL of the inhibitor, 0.1 mM NADPH, 0.1 M sodium phosphate buffer (pH 6.2), 10 mM of sodium D-glucuronate and enzyme to make a total volume of 200 μL . The reaction was initiated by the addition of substrate after the incubation for 10 min at 37 $^{\circ}\text{C}$. The change in absorbance was measured on a microplate at a wavelength of 340 nm after 5 min of incubation at 37 $^{\circ}\text{C}$. Correction of oxidation associated with NADPH was employed by appropriate blank while 20 μL of valproic acid (10 mM) was used as the positive control. A unit of the enzyme is defined as an amount of it required in catalyzing the oxidation of 1 mM of NADPH per minute in the assay condition.

ALR2 enzyme assay: ALR2 activity was assayed according to the prescribed method³⁹, the reaction mixture contained 40 μL of 10 mM DL-glyceraldehyde (substrate) and all other reagents were added in the way according to the ALR1 assay procedure. More so, 20 μL of quercetin (1 mM) was used as the positive control. All the reactions were performed in triplicate. The absorbance was recorded and the data was analyzed using PRISM 5.0 (GraphPad, San Diego, California, USA) to calculate the IC₅₀ value of the test sample which percentage inhibition is above 50 %. The percentage inhibition was calculated using the equation below, $100 - (\text{OD}_{\text{testwell}}/\text{OD}_{\text{control}}) \times 100$.⁴⁰

Molecular Docking

Molecular docking study of compound **3a** was performed using LeadIT from BioSolveIT, GmbH Germany.⁴¹ The compound structure was drawn using ACD/ChemSketch⁴² and was 3D optimized. Crystal structure of human aldose reductase PDB ID 1US0 was downloaded from RCSB protein data bank,⁴³ while crystal structure of porcine aldehyde reductase PDB ID 3FX4 was downloaded to carry out docking studies.

Using 'Load or Prepare' utility of LeadIT software, the receptor structures were prepared. In case of ALR-1, Nicotinamide adenine dinucleotide phosphate (NADP) was selected as a co-factor, while in case of ALR-2, Dihydro nicotinamide adenine dinucleotide phosphate (NADPH) was selected. Active site of the receptor was defined as the amino acid residues in 7.5 Å radii of the co-crystallized reference ligand. The co-crystallized reference ligand in case of ALR-1 was FX4 ([[(5Z)-5-{{3-(carboxymethoxy)-4-methoxyphenyl}methylidene}-2,4-dioxo-1,3-thiazolidin-3-yl]acetic acid) while in case of ALR-2, the reference ligand was

IDD594 (2-(2-[[4-bromo-2-fluorophenyl)methyl]carbamothioyl]-5-fluorophenoxy)acetic acid).

Using default docking parameters of the software, molecular docking studies were carried out. The docking parameters were first optimized by re-docking of the reference ligands and determining their RMSD values with the co-crystallized structures. After successful re-docking of the reference ligand, molecular docking of compound **3a** was carried out. Top ranking 30 poses of the compound **3a** was generated. The putative binding mode of the compound was then carefully selected after visual as well as HYDE assessment⁴⁴ and 3D docking figure of the compound was then prepared using Discovery Studio Visualizer v4.0.⁴⁵

Acknowledgment. Financial support by the Volkswagenstiftung (Az86223) and by the State of Mecklenburg-Vorpommern is gratefully acknowledged.

References

- (1) A. Strecker. *Justus Liebigs Ann. Chem* **1850**, 27–45.
- (2) a) Blaser, H.-U.; Federsel, H.-J., Eds. *Asymmetric Catalysis on Industrial Scale*; Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, Germany, 2010; b) Duthaler, R. O. *Tetrahedron* **1994**, *50*, 1539–1650; c) Patterson, D. E.; Xie, S.; Jones, L. A.; Osterhout, M. H.; Henry, C. G.; Roper, T. D. Large-Scale Application of Asymmetric Phase-Transfer Catalysis for Amino Acid Synthesis. In *Asymmetric Catalysis on Industrial Scale*; Blaser, H.-U.; Federsel, H.-J., Eds.; Wiley-VCH Verlag GmbH & Co. KGaA: Weinheim, Germany, 2010, pp 473–484; d) Weiner, B.; Szymański, W.; Janssen, D. B.; Minnaard, A. J.; Feringa, B. L. *Chem. Soc. Rev.* **2010**, *39*, 1656;
- (3) a) Aceña, J. L.; Sorochinsky, A. E.; Moriwaki, H.; Sato, T.; Soloshonok, V. A. *Journal of Fluorine Chemistry* **2013**, *155*, 21–38; b) Nádvorník, M.; Langer, V.; Jirásko, R.; Holčapek, M.; Weidlich, T.; Lyčka, A.; Popkov, A. *Polyhedron* **2008**, *27*, 3477–3483; c) Popkov, A.; Spiegeleer, B. de. *Dalton Trans* **2012**, *41*, 1430–1440; d) Soloshonok, V.; Ueki, H.; Ellis, T. *Synlett* **2009**, *2009*, 704–715; e) Sorochinsky, A. E.; Aceña, J. L.; Moriwaki, H.; Sato, T.; Soloshonok, V. *Amino Acids* **2013**, *45*, 1017–1033; f) Wang, J.; Zhang, L.; Jiang, H.; Chen, K.; Liu, H. *Chimia (Aarau)* **2011**, *65*, 919–924;

- (4) a) Soloshonok, V. A.; Cai, C.; Hruby, V. J. *Tetrahedron: Asymmetry* **1999**, *10*, 4265–4269; b) Soloshonok, V. A.; Cai, C.; Hruby, V. J.; van Meervelt, L.; Yamazaki, T. *J. Org. Chem.* **2000**, *65*, 6688–6696;
- (5) Soloshonok, V. A.; Avilov, D. V.; Kukhar, V. P.; van Meervelt, L.; Mischenko, N. *Tetrahedron Letters* **1997**, *38*, 4671–4674.
- (6) a) Soloshonok, V. A.; Kukhar, V. P.; Galushko, S. V.; Svistunova, N. Y.; Avilov, D. V.; Kuz'mina, N. A.; Raevski, N. I.; Struchkov, Y. T.; Pysarevsky, A. P.; Belokon, Y. N. *J. Chem. Soc., Perkin Trans. 1* **1993**, 3143; b) Soloshonok, V. A.; Avilov, D. V.; Kukhar, V. P.; Tararov, V. I.; Savel'eva, T. F.; Churkina, T. D.; Ikonnikov, N. S.; Kochetkov, K. A.; Orlova, S. A.; Pysarevsky, A. P.; Struchkov, Y. T.; Raevsky, N. I.; Belokon', Y. N. *Tetrahedron: Asymmetry* **1995**, *6*, 1741–1756;
- (7) Sorochinsky, A. E.; Aceña, J. L.; Moriwaki, H.; Sato, T.; Soloshonok, V. A. *Amino Acids* **2013**, *45*, 691–718.
- (8) Houck, D.; Luis Aceña, J.; Soloshonok, V. A. *HCA* **2012**, *95*, 2672–2679.
- (9) a) Chinchilla, R.; Nájera, C. *Chem. Rev.* **2007**, *107*, 874–922. b) Chinchilla, R.; Nájera, C. *Chem Soc Rev* **2011**, *40*, 5084–5121. c) Thomas, A. M.; Sujatha, A.; Anilkumar, G. *RSC Adv.* **2014**, *4*, 21688.
- (10) Dibowski, H.; Schmidtchen, F. P. *Angew. Chem. Int. Ed.* **1998**, *37*, 476–478.
- (11) a) Brea, R. J.; López-Deber, M. P.; Castedo, L.; Granja, J. R. *The Journal of organic chemistry* **2006**, *71*, 7870–7873; b) Crisp, G. T.; Gore, J. *Tetrahedron* **1997**, *53*, 1523–1544; c) Crisp, G. T.; Robertson, T. A. *Tetrahedron* **1992**, *48*, 3239–3250; d) Greenspan, P. D.; Clark, K. L.; Tommasi, R. A.; Cowen, S. D.; McQuire, L. W.; Farley, D. L.; van Duzer, John H.; Goldberg, R. L.; Zhou, H.; Du, Z.; Fitt, J. J.; Coppa, D. E.; Fang, Z.; Macchia, W.; Zhu, L.; Capparelli, M. P.; Goldstein, R.; Wigg, A. M.; Doughty, J. R.; Bohacek, R. S.; Knap, A. K. *J. Med. Chem.* **2001**, *44*, 4524–4534; e) Pérez-Alvite, M. J.; Mosquera, M.; Castedo, L.; Granja, J. R. *Amino acids* **2011**, *41*, 621–628; f) Shchetnikov, G. T.; Zotova, M. A.; Bruneau, C.; Dixneuf, P. H.; Osipov, S. N. *Eur. J. Org. Chem.* **2010**, *2010*, 1587–1592;
- (12) Zimmet, P.; Alberti, K. G.; Shaw, J. *Nature* **2001**, *414*, 782–787.
- (13) El-Kabbani, O.; Ruiz, F.; Darmanin, C.; Chung, R. P.-T. *Cellular and molecular life sciences : CMLS* **2004**, *61*, 750–762.
- (14) Kubo, E.; Miyoshi, N.; Fukuda, M.; Akagi, Y. *Experimental Eye Research* **1999**, *68*, 457–464.

- (15) Lee, A. Y.; Chung, S. S. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology* **1999**, *13*, 23–30.
- (16) Kador, P. F.; Kinoshita, J. H.; Tung, W. H.; Chylack Jr., L. T. *Invest. Ophthalmol. Vis. Sci.* **1980**, *19*, 980–982.
- (17) Srivastava, S.; Spite, M.; Trent, J. O.; West, M. B.; Ahmed, Y.; Bhatnagar, A. *The Journal of biological chemistry* **2004**, *279*, 53395–53406.
- (18) Tabakoff, B.; Erwin, V. G. *J. Biol. Chem.* **1970**, *245*, 3263–3268.
- (19) Tulsiani, D. R.; Touster, O. *J. Biol. Chem.* **1977**, *252*, 2545–2550.
- (20) Hara, A.; Deyashiki, Y.; Nakayama, T.; Sawada, H. *Eur J Biochem* **1983**, *133*, 207–214.
- (21) Sawada, H.; Hara, A.; Nakayama, T.; Kato, F. *Journal of biochemistry* **1980**, *87*, 1153–1165.
- (22) Wermuth, B.; Münch, J. D.; von Wartburg, J P. *The Journal of biological chemistry* **1977**, *252*, 3821–3828.
- (23) Dunlop, M. *Kidney Int* **2000**, *58*, 3–12.
- (24) Yabe-Nishimura, C. *Pharmacological reviews* **1998**, *50*, 21–33.
- (25) Lipinski, C. A.; Lombardo, F.; Dominy, B. W.; Feeney, P. J. *Adv. Drug Deliv. Rev* **2012**, *64*, 4-17
- (26) Sander, T., OSIRIS property explorer. Allschwil: Actelion Pharmaceuticals Ltd 2001.
<http://www.organic-chemistry.org/prog/peo/index.html>
- (27) Carbone, V.; Giglio, M.; Chung, R.; Huyton, T.; Adams, J.; Maccari, R.; Ottana, R.; HARA, A.; El-Kabbani, O. *European journal of medicinal chemistry* **2010**, *45*, 1140–1145.
- (28) Barski, O. A.; Gabbay, K. H.; Bohren, K. M. *Genomics* **1999**, *60*, 188–198.
- (29) El-Kabbani, O.; Carbone, V.; Darmanin, C.; Oka, M.; Mitschler, A.; Podjarny, A.; Schulze-Briese, C.; Chung, R. P.-T. *Journal of medicinal chemistry* **2005**, *48*, 5536–5542.
- (30) El-Kabbani, O.; Sthanam, L.; Narayana, V. L.; Moore, K. M.; Green, N. C.; Flynn, T. G.; Delucas, L. J. *Acta crystallographica. Section D, Biological crystallography* **1993**, *49*, 490–496.
- (31) Del Corso, A.; Costantino, L.; Rastelli, Buono, G. F.; Mura, U. *Exp. Eye Res* **2000**, *71*, 515–521
- (32) El-Kabbani, O.; Wilson, D. K.; Petrash, J. M.; Quiocho, F. A. *Mol. Vis.* **1998**, *4*, 19-25
- (33) Hayman, S.; Kinoshita, J. H. *J. Biol. Chem.* **1965**, *240*, 877.

- (34) El-Kabbani, O.; Darmanin, C.; Schneider, T. R.; Hazemann, I.; Ruiz, F.; Oka M. *Proteins*. **2004**, 55(4):805-813
- (35) Carbone, V.; Chung, R.; Endo, S.; Hara, A.; El-Kabbani, O. *Arch. Biochem. Biophys.* **2008**, 479(1):82-87.
- (36) Daly, A. K.; Mantle, T. J. *J. Biochem.* **1982**, 205, 373–380.
- (37) Kador, P. F.; Kinoshita, J. H.; Brittain, D. R.; Mirrlees D. J.; Sennitt, C. M.; Stribling, D. *J. Biochem.* **1986**, 240, 233–237.
- (38) Costantino, L.; Rastelli, G.; Gamberini, M. C.; Vinson, J. A.; Bose, P.; Iannone, A.; Staffieri, M.; Antolini, L.; Del Corso, A.; Mura, U.; Albasini, A. *Journal of medicinal chemistry* **1999**, 42, 1881–1893.
- (39) Da Settimo, F.; Primofiore, G.; La Motta, C.; Salerno, S.; Novellino, E.; Greco, G.; Lavecchia, A.; Laneri, S.; Boldrini, E. *Bioorganic & medicinal chemistry* **2005**, 13, 491–499.
- (40) Saraswat, M.; Muthenna, P.; Suryanarayana, P.; Petrash, J. M.; Reddy, G. B. *Asia Pacific journal of clinical nutrition* **2008**, 17, 558–565.
- (41) LeadIT: BioSolveIT GmbH, Germany; **2014**.
- (42) ACD/ChemSketch. 14.00 ed: Advanced Chemistry Development, Inc., Toronto, On, Canada; **2015**.
- (43) Berman H. M., Westbrook J., Feng Z., Gilliland G., Bhat T., Weissig H. *Nucleic Acids Res.* **2000**, 28(1), 235-242
- (44) Schneider N., Lange G., Hindle S., Klein R., Rarey M. J. *Comput. Aided. Mol. Des.*, **2013**, 27(1):15-29.
- (45) Discovery StudioVisualizer. 4.0 ed: Accelrys Software Inc; **2005**.

