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Asymmetric Reduction of 3-Ketoproline Ethyl Ester by Modified Borohydrides and Various Vegetables

Agustono Wibowo, Zurina Shaameri, Mohd Fazli Mohammat, Ahmad Sazali Hamzah*
*Organic Synthesis Laboratory, Institute of Science, Universiti Teknologi MARA (UiTM), 42300 Bandar Puncak Alam, Selangor Darul Ehsan, Malaysia. *E-mail: asazali@salam.uitm.edu.my*
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ABSTRACT. Reduction of (±)-3-ketoproline ethyl ester (**1**) by $NaBH_4$ in the presence of $CaCl_2$ and $MgCl_2$ as the chelating agents gave selective products *cis*-3(*R*/*S*)-alcohols, while reduction by $NaBH_4$ alone or chelated with $NiCl_2$ and $AlBr_3$ gave mixtures of *cis*- and *trans*-alcohols. The reduction of (±)-**1** by various vegetables however, gave exclusively the *cis*-alcohol as the major and *trans*-alcohol as the minor. On the contrary, reduction of (±)-**1** by carrot afforded a mixture of *cis*- and *trans*-alcohols, in which the *trans*-alcohol exists as the major product. In addition, we found that this biocatalyst selectively converted *S*-enantiomer of (±)-**1** to the *cis*-alcohol, and *R*-enantiomer to a mixture of *cis*- and *trans*-alcohols with *cis*-alcohol as the major product. This fact prompted us to use various fresh plant tissues for stereoselective reduction of diverse types of pyrrolidinones, as its stereoselectivity towards racemic mixtures is higher compared to that using chemical reducing agents.

Key words: Ketoproline, Stereoselective reduction, Vegetable, Sodium borohydride, Metal salt

INTRODUCTION

Pyrrolidine ring is one of the most important groups in medicinal chemistry,[1–3] as it is contained in several important drugs in the market such as two hypotensors Captopril and Zofenopril,[4,5] antidiabetic Vildagliptin,[6] and antidepressant Sulpiride.[7] Recently, several new drug candidates of proline derivatives, such as derivatives of lactacystin (**2**) [omuralide (**3**) and PS-159 (**4**)], salinosporamide A **5**, and BMS-564,929 (**6**) are currently in clinical trials. Pyrrolidinone **2** significantly inhibits the proliferation of C6 cells, increases apoptosis and reduced mitochondrial membrane potential for the treatment option for glioma,[8] proline **4** is being studied for the treatment of reperfusion injury that occurs following cerebral ischemia and myocardial infarction,[9] prolines **3** an **5** is a potent proteasome inhibitor for the treatment of various cancers,[10,11] and pyrrolidinone **6** is an investigational selective androgen receptor modulator for the treatment of the symptoms of age-related decline in androgen levels in men (andropause).[12] These symptoms may include depression, loss of muscle mass and strength, reduction in libido and osteoporosis.[13]

The presence of hydroxyl substituent at C-3 on the pyrrolidine rings of **2** and **6** is essential, of which both (*S*)-OH or (*R*)-OH enantiomers are responsible for their activity as mentioned above (*Fig.* 1).[11,14] A number of synthetic approaches towards **2** and **6** have been reported,[15–17] and several synthetic works have been reviewed.[11,18] In some approaches, one of the important routes towards skeletons **2** and **6** is asymmetry reduction of the prochiral ketone at the C-3 position of the chiral alcohol. Customarily, asymmetry reduction of pyrrolidine-type of compound is performed using boron-based reducing agents [$NaBH(OAc)_3$ and $NaBH_4$] or transition metals (Ru^{2+} and Ir^{2+}) complexed with chiral ligands,[19,20] while bioreduction is accomplished using Baker's yeast.[21] More recently, vegetables were reported to have

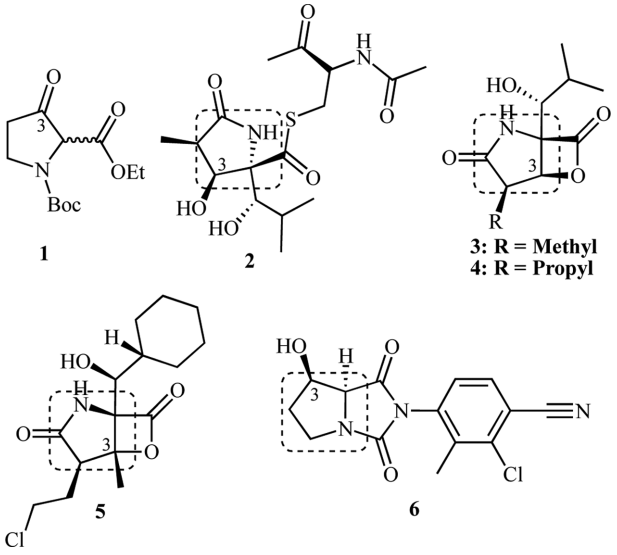


***Figure* 1.** Chemical structures of new drug candidates containing proline skeleton (**2**–**4**) and an analogue *N*-boc-3-ketoproline ethyl ester (**1**).

the ability to reduce several types of ketones (e.g. aliphatic and aromatic ketone, cyclic ketone, β-ketoesters and azidoketone) with excellent stereoselectivity.[22] Reduction employing such vegetables could be one of the alternative stereoselective protocols, as it has been reported to be very efficient and performed under mild conditions. Furthermore, the reduced products are often easily purified with high regio- and stereoselectivity.[23,24]

Hydroxyprolines are important intermediates from pyrrolidine derivatives used to prepare various kinds of chiral pharmaceuticals and chiral antibiotics.[25,26] This proline derivative could be prepared from direct reduction of its respective keto form (ketoproline). To date, asymmetry reductions of ketoproline-types (cyclic β-ketoester) to hydroxyprolines using biological catalysts other than the Baker's yeast, and asymmetry reductions using $NaBH_4$ complexed with metal salts have never been reported. Previously, reduction of cyclic β-ketoester-types using $NaBH_4$ complexed with metal salts had been reported by Fraga et al.,[27] however it was only on cyclopentanones, butyrolactones and cyclohexanones. Hence, in continuation to our interests in the study of pyrrolidine rings,[28–33] we report herein the stereospecific reductions of a proline derivative, *N*-boc-3-ketoproline ethyl ester (**1**) using sodium borohydride in the presence or absence of metal salts as the chelating agents, as well as by different vegetables.

EXPERIMENTAL

General Information

ECD spectrum and optical rotation were recorded on a spectropolarimeter (JASCO J-720WI) and Anton-Paar MCP200 Polarimeter. High-resolution mass spectra were obtained from Mass Spectrometry Instruments (MSI), Autoconcept-HRMS with MACH 3Xe data system. The ^1H and ^{13}C NMR spectra were registered in $CDCl_3$ with Joel Resonance ECZ400S [400 MHz (^1H) and 100 MHz (^{13}C)] using TMS as the internal standard. Preparative thin layer chromatography (PTLC) was done using silica gel 60 F_{254} pre-coated glass sheets (layer thickness 2 mm, Merck). Analytical TLC was performed on silica gel 60 F_{254}, Merck (layer thickness 0.25 mm, Merck) and visualized with UV light and $KMnO_4$ as the detecting agent. Enantiomeric excesses were determined by HPLC analysis on Agilent 1260 Infinity Quaternary LC equipped with a UV detector and a chiral column Chiralpak AS-H (4.5 mm × 250 mm). (±)-*N*-boc-3-ketoproline ethyl ester (**1**) were prepared from ethyl glycine hydrochloride and ethyl acrylate according to the procedure previously described by Williams *at al*.[34]. The enantiomeric mixture of **1** (%*ee* 2.6:1) was determined by chiral column Chiralpak AS-H.

Reduction of *N*-boc-3-ketoproline ethyl ester (1) using $NaBH_4$ in the presence or absence of metal salts

A solution of substrate **1** (100 mg) in methanol, in the presence or absence of anhydrous $CaCl_2$, $MgCl_2$, $NiCl_2$ and $AlCl_3$ (2 eq) was stirred at room temperature for 30 min. The reaction mixture was cooled at 0 °C, and $NaBH_4$ (1.2 eq) was slowly added. After being stirred for another 30 min, the bulk of methanol was removed in vacuo. The resulting mixture was poured into saturated NH_4Cl solution and extracted with ethyl acetate (50 mL × 3). The organic layer was dried over anhydride Na_2SO_4 and concentrated in vacuo. Purification of the product by preparative TLC (eluted with *n*-hexane-ethyl acetate 8:2) provide a mixture of *cis*- and *trans*-3-hydroxyproline ethyl ester as summarized in *Table* 1. Identification and semi-preparative isolation of the enantio-products of alcohols **1a**, **1b**, **1c** and **1d** were performed on the chiral column (Chiralpak AS-H). The solvent system was *n*-hexane : ethyl acetate : methanol (60:35:5), a flow rate of 0.5 mL/min and wavelength of 280 nm. Enantiomeric excess (*ee*) and diastereomeric excess (*de*) were determined directly chiral column HPLC, while its absolute configurations were established using CD spectra.

Ketoproline (1): Pale yellow oil, ^1H NMR (400 MHz,

***Table* 1.** Reduction of *N*-boc-3-ketoproline ethyl ether (**1**) by $NaBH_4$ and $NaBH_4$-Metal salts

Reagent	Yield (%)[a]		*de* (%)[b]		*ee* (%)[b]	
	cis	*trans*	**1a**	**1c**	**1a**	**1b**
$NaBH_4$	78.7	6.4	92.8	92.1	64.9	62.6
$NaBH_4$ - $MgCl_2$	75.5	-	100	100	66.2	-
$NaBH_4$ - $CaCl_2$	48.6	-	100	100	63.7	-
$NaBH_4$ - $NiCl_2$	49.2	2.2	95.8	95.5	65.8	64.1
$NaBH_4$ - $AlBr_3$	7.3	1.2	87.4	83.6	65.4	58.1

Config: absolute configuration; *de*: diastereomeric excess; *ee*: enantiomeric excess; [a]Method: Column chromatography (Hexane:$CHCl_3$: Acetone=3:6:1); [b]Method: Relative intensity by chiral column HPLC peak area.

$CDCl_3$, 25 °C): δ (enantiomeric mixtures; 2.6:1) = 4.30 and 4.25 (s, 1H), 4.02 (q, *J* = 7.2 MHz, 2H), 3.66 (m, 1H), 3.58 (m, 1H), 2.49 (m, 2H), 1.28 and 1.21 (s, 9H) and 1.09 (t, *J* = 6.8 MHz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ (enantiomeric mixtures; 2.6:1) = 204.7/204.2, 166.3/166.1, 154.1/158.8, 81.0, 65.8/65.4, 62.1, 42.2/41.6, 37.1/36.4, 27.8 and 14.2.

Hydroxyproline (1a): Colorless oil, $[\alpha]^{20}_D$ = −31.59 (*c* = 0.38 μM, MeOH); ^{1}H NMR (400 MHz, Acetone-d_6, 25 °C): δ 4.59 (br s, 1H), 4.32 (d, *J* = 6.8 MHz, 1H), 4.21 (q, *J* = 6.4 MHz, 2H), 3.63 (m, 1H), 3.48 (m, 1H), 2.09 (m, 1H), 1.99 (m, 1H), 1.40 (s, 9H) and 1.29 (t, *J* = 6.8 MHz, 3H). ^{13}C NMR (100 MHz, Acetone-d_6): δ 170.2, 153.7, 79.1, 71.9, 63.9, 60.2, 43.8, 31.9, 27.7 and 14.0. CD (*c* = 0.38 μM, MeOH) nm [Δε]: 212 [−0.90], 238 [−0.27], 259 [−0.03], 270 [−0.08], 289 [0.08], 304 [−0.08] and 317 [0.03].

Hydroxyproline (1b): Colorless oil, $[\alpha]^{20}_D$ = +10.35 (*c* = 0.38 μM, MeOH); ^{1}H NMR (400 MHz, Acetone-d_6, 25 °C): δ 4.43 (br s, 1H), 4.26 (s, 1H), 4.17 (q, *J* = 7.6 MHz, 2H), 3.60 (m, 2H), 2.11 (m, 1H), 1.95 (m, 1H), 1.45 (s, 9H) and 1.28 (t, *J* = 6.8 MHz, 3H). ^{13}C NMR (100 MHz, Acetone-d_6): δ 170.8, 153.7, 78.8, 74.4, 68.1, 61.4, 44.6, 32.7, 28.5, and 14.2.

Hydroxyproline (1c): Colorless oil, $[\alpha]^{20}_D$ = +10.53 (*c* = 0.38 μM, MeOH); ^{1}H NMR (400 MHz, Acetone-d_6, 25°C): δ 4.57 (br s, 1H), 4.39 (d, *J* = 6.4 MHz, 1H), 4.23 (q, *J* = 6.4 MHz, 2H), 3.62 (m, 1H), 3.46 (m, 1H), 2.24 (m, 1H), 1.17 (m, 1H), 1.44 (s, 9H) and 1.27 (t, *J* = 6.8 MHz, 3H). ^{13}C NMR (100 MHz, Acetone-d_6): δ 170.0, 154.0, 79.0, 71.0, 63.5, 60.1, 44.2, 32.5, 27.8 and 13.9.

Hydroxyproline (1d): Colorless oil, $[\alpha]^{20}_D$ = −21.06 (*c* = 0.38 μM, MeOH); ^{1}H NMR (400 MHz, Acetone-d_6, 25 °C): δ 4.43 (br s, 1H), 4.18 (q, *J* = 7.6 MHz, 2H), 4.15 (s, 1H), 3.63 (m, 2H), 2.08 (m, 1H), 1.92 (m, 1H), 1.40 (s, 9H) and 1.27 (t, *J* = 6.8 MHz, 3H). ^{13}C NMR (100 MHz, Acetone-d_6): δ 171.4, 153.5, 79.1, 71.9, 63.9, 60.2, 43.8, 31.9, 27.7 and 14.4. CD (*c*=0.38 μM, MeOH) nm [Δε]: 216 [−0.8], 258 [−0.25], 288 [−0.09], 302 [−0.16] and 322 [0.07].

Biocatalyzed reduction using vegetables

Fresh sample of vegetables were rinsed with 5% sodium hypochlorite solution and sterile distilled water, and cut into small pieces (approx. 1 cm long slice) with a sterile knife. In separate experiments, substrate **1** (100 mg) was added to the suspension of vegetable slices (10 g) in 70 mL of distilled water. The mixture was treated by shaking (120 rpm) at room temperature. Finally, the suspension was filtered off, and the vegetables slices were washed with distilled water. The aqueous solution was then extracted with ethyl acetate (3 × 50 mL), and the organic phase was dried with anhydrous Na_2SO_4 and evaporated *in vacuo*. The resulting residue was applied to a chiral column HPLC (Chiralpak AS-H). The solvent system was *n*-hexane : ethyl acetate : methanol (60:35:5), a flow rate of 0.5 mL/min and wavelength of 280 nm. Enantiomeric excess (*ee*) and diastereomeric excess (*de*) were determined directly from the areas under the curve of the alcohols 1a, 1b, 1c and 1d as summarized in *Table* 2.

***Table* 2.** Biocatalytic reduction of racemic *N*-boc-3-ketoproline ethyl ester (**1**) by various vegetables[a]

No	Biocatalysts	Inc. (days)	Conv. Yield[b] (%)	Conv.[b]		*de* (%)[b]	
				(*S*)-**1**	(*R*)-**1**	**1a**	**1c**
1	Cucumber (*Cucumis sativus*)	7	18.9	100	18.9	100	94.5
2	Calabash (*Cucurbita lagenaria* L.)	15	1.5	99.4	2.1	100	100
3	Bitter gourd (*Momordica charantia*)	7	58.9	100	58.9	100	95.3
4	Carrot (*Daucus carota* L.)	2	94.5	94.4	100	100	2.1
5	Winter radish (*Raphanus sativus* L.)	21	1.9	-	4.7	-	96.4
6	Red radish (*Raphanus sativus* L.)	15	7.8	94.1	13.6	100	95.7
7	Potato (*Solanum tuberosum* L.)	7	18.3	100	18.3	100	91.9
8	Chinese yam (*Dioscorea polystachya*)	7	11.5	100	11.5	100	67.9
9	Yacon root (*Smallanthus sonchifolius*)	21	4.8	100	4.8	100	100
10	Jicama (*Pachyrhizus erosus*)	3	64.8	95.6	69.2	100	91.0
11	Cassava (*Manihot esculenta*)	7	11.4	98.4	13.0	100	98.8
12	Red sweet potato (*Ipomoea batatas* L.)	2	24.8	100	24.8	100	97.8
13	Turmeric (*Curcuma longa* L.)	21	24.3	100	24.3	100	76.8
14	Ginger (*Zingiber officinale*)	2	56.1	94.4	61.7	100	93.9
15	Galangal (*Alpinia galanga*)	7	15.0	100	15.0	100	90.6
16	Garlic (*Allium sativum*)	7	59.2	100	59.1	100	100
17	Onion (*Allium cepa*L.)	21	6.9	100	6.9	100	100

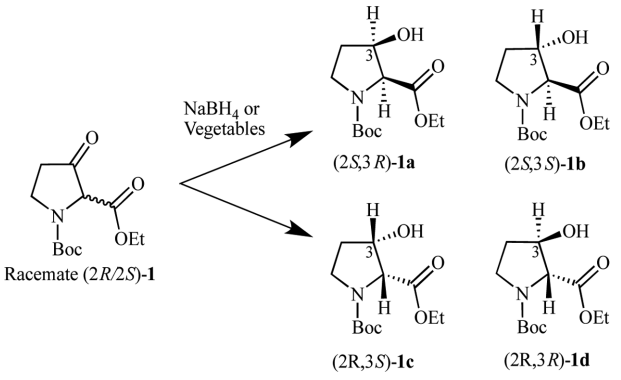
RESULTS AND DISCUSSION

Reduction of (±)-*N*-boc-3-ketoproline ethyl ester (1) by $NaBH_4$ reagent

It is known that aldehydes and ketones are rapidly reduced by $NaBH_4$. In addition, combinations with metal salts have also been reported increasing the yield and selectivity of reduced products of various kinds of prochiral ketones.[35] Nevertheless, this method provides a simple inexpensive alternative procedure, which proceeds under mild condition, neutral pH and short reaction time.[36] Thus, in this work, we had used $NaBH_4$ alone and together with several metal salts ($CaCl_2$, $MgCl_2$, $NiCl_2$ and $AlBr_3$) to reduce (±)-*N*-boc-3-ketoproline ethyl ester (**1**) that would lead to the desired product that will be used as precursor in the synthesis of lactacystin analogues.

Initially, ketoproline **1** in methanol was treated with 1.2 molar equivalent $NaBH_4$, or with 2 molar equivalent of metal salts; $CaCl_2$, $MgCl_2$, $NiCl_2$ and $AlBr_3$ followed by 2 molar equivalent $NaBH_4$ reagent. After work-up, the mixtures of alcohol products (*Scheme* 1) were purified by column chromatography, and followed by chiral column HPLC to determine the ratio of % *ee* and absolute configuration of the alcohol products (*Table* 1).

The structure of all alcohol products **1a-1d** have been interpreted based on the ^1H and ^{13}C NMR spectral data, while the configuration of protons H-2 and H-3 at the chiral carbons of alcohols **1a**/**1c** and alcohols **1b**/**1d** as *cis* and *trans*, respectively, have been confirmed from the NOE correlation. Moreover, further study using Electronic Circular Dichroism (ECD) spectra data established that configurations of the chiral carbon C-2 in alcohols **1a** and **1d** were *S* and *R*, respectively (*Fig.* 2). With respect to the ECD spectra and optical rotation data of alcohols **1a** and **1d**, it could be concluded that configurations of alcohols **1b** and **1c** were 2*R* and 2*S*, respectively.



***Scheme* 1.** Reduction products of (**1**).

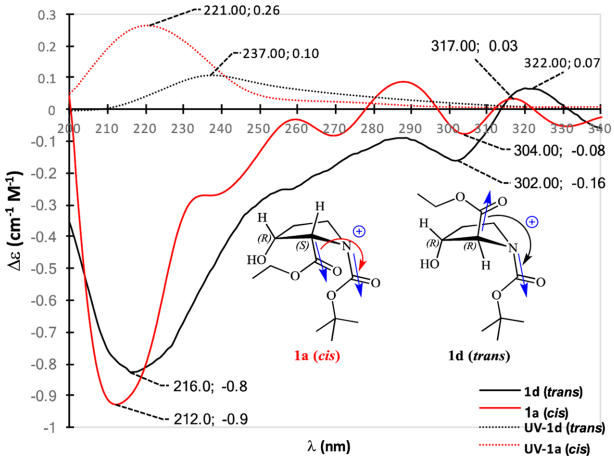


***Figure* 2.** Experimental CD and UV Spectral of **1a** and **1d**.

Reduction of **1** with $NaBH_4$ produced 78.7% of the *cis*-alcohol and 6.4% of the *trans*-alcohol in mixtures of enantiomers (64.9% *ee* and 62.6% *ee*, respectively) (*Table* 1). Adding metal chlorides $CaCl_2$ and $MgCl_2$ increased the selectivity of reduction towards only the *cis*-alcohol with 100% *de*. In the presence of metal chloride $NiCl_2$, the proportion of the *cis*-alcohol increased from 92.8% *de* to 95.5% *de*. Nevertheless, with metal bromide $AlBr_3$, the reaction did not go towards completion (*cis*-alcohol 7.3% yield, *trans*-alcohol 1.2% yield), and increasing the amount of reagent or prolonging the reaction time had no effect. In this study, using the combination $NaBH_4$-metal salt decreased the yield of the alcohol products compared to using $NaBH_4$ alone. Besides that, both (*R*)- and (*S*)-enantiomers of **1** reduced to their corresponding *cis*-alcohols with the same feasibility, as % *ee* of products were consistent with the enantiomeric ratio of the starting material **1** (*ee* 2.6:1).

Steric hindrance of the C-3 keto groups in (*S*)- and (*R*)-enantiomers of **1** by the C-2 ester group led to *Si*-face and *Re*-face attack by $NaBH_4$ (*Fig.* 3); consequently, the *cis*-alcohol (2*S*,3*R*)-**1a** (92.8% *de*) and (2*R*,3*S*)-**1c** (92.1% *de*) were formed, respectively (*Scheme* 1). This effect was strengthened by the addition of metal salts, as the *cis*-alcohol product was formed in a higher *de*. However, the opposite result was observed for the combination of $NaBH_4$ with metal salt $AlBr_3$ (**1a** 87.4% *de* and **1c** 83.6% *de*), in which the selectivity was lower than that when used $NaBH_4$ alone. Based on its ionic radius, the results of this study displayed that, the reduction of 3-ketoproline ethyl ester (**1**) with $NaBH_4$ in the presence of metal chloride did not show a direct correlation between ionic radius and the diastereoselectivity. This is contrary to the results previously described by Taniguchi *et al*.,[37] in which the selectivity reduction decreased

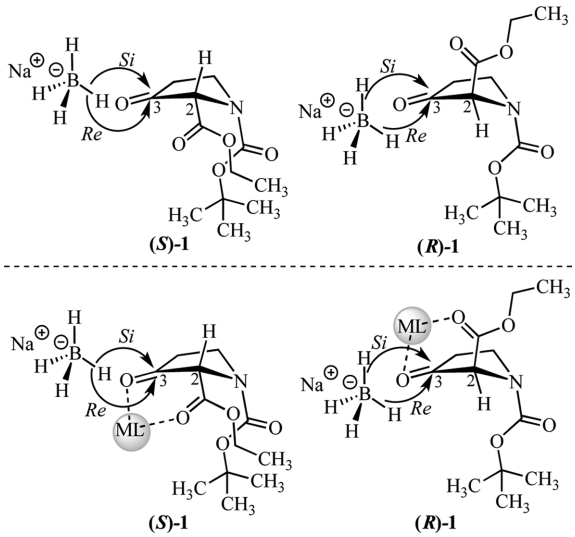


***Figure* 3.** The mechanism of action of $NaBH_4$ (upper) and $NaBH_4$-Metal salt (bottom) in reducing ketoproline (**1**).

along with the increasing of ionic radius. However, the result was similar to that reported by Fraga et al.,[27] on the reduction of cyclic β-ketoester. This might due to the different properties of cyclic β-ketoester and *straight-chain* β-ketoester. The ionic radius of metal and the ratio of the products (*cis*/*trans*) were as follows: Ca^{2+} 1.14 Å (100%); Mg^{2+} 0.86 Å (100%); Ni^{2+} 0.74 Å (95.8/4.2); Al^{3+} 0.67 Å (87.4/12.6). The % *de* and % *ee* of the reduction products of (±)-**1** are shown in *Table* 1.

Biocatalytic reduction of (±)-*N*-boc-3-ketoproline ethyl ester (1) by vegetables

Whole plant tissue is an excellent choice to substituted isolated enzymes, since the oxido-reductase, cofactor and the cofactor regeneration systems are all within the cells.[38,39] Thus reductants being eco-friendly environmental reducing agents, accessible in all seasons and have the advantage of a being low cost. Therefore, to find an ideal biocatalyst for asymmetric reduction reaction of the (±)-*N*-boc-3-ketoproline ethyl ester (**1**), several vegetables as summarized in *Table* 2 were applied as the biocatalysts.

As shown in *Table* 2, the incubation of ketoproline **1** with fresh vegetables in water at room temperature gave its corresponding alcohol (*Scheme* 1) with high product enantioselectivity. In particular, the (*S*)-enantiomer of **1** was completely reduced affording a single diastereomer, *cis*-alcohol **1a** (100% *de*), whilst the (*R*)-enantiomer was reduced to a mixture of diastereomers, *cis*-alcohol **1c** (> 90% *de*) and *trans*-alcohol **1d** (< 10% *de*). The absence of *trans*-alcohol **1b** in the former reaction product indicates that the vegetables catalytically reduced (±)-**1** in a diastereospecific manner. Furthermore, the low conversion of the (*R*)-enantiomer shows that those biocatalysts had good enantioselectivity towards the racemic substrates, which the (*S*)-enantiomer was reduced before the (*R*)-enantiomer. This case is similar to the reduction of two β-ketoester racemates (ethyl 2-oxo-1-cyclopentane-carboxylate and ethyl 2-oxo-1-cyclohexane-carboxylate) by *Daucus carota* (carrot), in which one of the enantiomers was reduced faster than the other.[24,40] Interestingly in the reduction of (*R*)-enantiomer via carrot afforded the *trans*-alcohol **1d** (97.9% *de*) as the major product and *cis*-alcohol **1c** (2.1% *de*) as the minor.

The transfer of a hydride (*Re*- or *Si*-faces) in the formation of (*R*)- and (*S*)-alcohols depends on the orientation of the substrate binds to the enzyme, while enzyme (dehydrogenase) transfers either pro-(*R*)-hydride or pro-(*S*)-hydride of the coenzymes (NADPH) depending on the kind of the enzyme.[41] Every plant species have the ability to produce several enzymes, which are able to distinguish between small and large groups, thus hydride is delivered from either the *Re*- or *Si*-face in an enantiomeric way.

The formation of diastereomer (2*S*,3*R*)-**1a** in high *de* indicated that the dehydrogenase pro-(*R*)-hydride in our selected vegetables was more dominant than its pro-(*S*)-hydride. Therefore, the dehydrogenase delivered the hydride exclusively from the *Si*-face of the carbonyl group of both enantiomers to give the (*R*)-alcohol. However, the selectivity of the dehydrogenase also depends on the size of the carbon chains attached to the carbonyl group.[42] *Fig.* 4 shows the possible mechanism action of transferring a hydride from NADPH to the carbonyl group of **1**. This mechanism mimics the (*R*)-2-hydroxyglutarate dehydrogenase catalysis reduction of 2-oxoglutarate to (*R*)-2-hydroxyglutarate as reported by Martins *et al*.[43] Based on this mechanism, the large steric hindrance around the carbonyl group of the (*S*)-enantiomer of **1** only allows the hydride to attack from the *Si*-face, thus only a single diastereomer which is *cis*-alcohol **1a** was detected. On the other hand, the less steric hindrance around the carbonyl group of the (*R*)-enantiomer of **1** allows the hydride to attack from both the *Re*- and *Si*-faces to give the mixture of *cis*- and *trans*-alcohols, with the attack from the *Re*-face is more favorable than that from the *Si*-face. However, in the reduction of (*R*)-enantiomer of **1** using carrots, the attack from the *Si*-face is more favorable and *trans*-alcohol is found to be the major product. This may be due to that the main hydride from the enzyme in the carrot is the dehydrogenase pro-(*R*)-hydride.

In this study, the selectivity reduction of all selected vegetables is used very high. Nevertheless, the long reduction

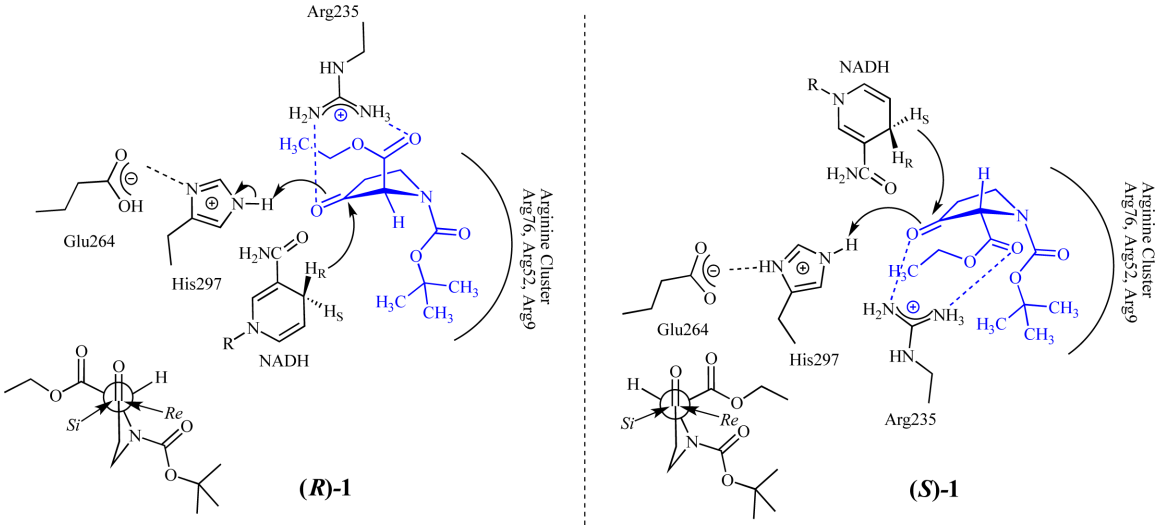


***Figure* 4.** Mechanism of an NADH-depending dehydrogenase transferring a hydride between substrate and coenzyme.[28]

times and low product yields are unfavorable and need to be optimized. In this case, Utsukihara *et al.*[44] has described that the problem using plant culture-cells as biocatalysts is that they usually grow very slow and require a large amount of the catalyst. However, according to Matsuo *et al.*[45] the addition of sucrose can shorten the reduction time; unfortunately this did not affect the chemical yield and enantioselectivity. The best result in this study was shown by carrot (94.5% yield in two days) and jicama (64.8% yield in three days), while the moderate results were displayed by ginger (56.1% yield in two days), bitter gourd (58.9% yield in seven days) and garlic (59.2% yield in seven days).

CONCLUSION

In conclusion, both $NaBH_4$-Metal and vegetable are useful reducing agents to produce chiral hydroxyproline. However, vegetable is more promising, as it has the ability to selectively reduce a racemic mixture. Besides that, the cheap and easily accessible vegetables as well as the ease of the work-up and eco-friendly reactant procedure, provide an attractive environmentally acceptable option, which could be used in an industrial scale. Future work in this study should be devoted to the current limitations concerning conversion yield, stereoselectivity and long reaction times.

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