Journal of Academia Vol. 10, Issue 1 (2022) 102 – 106

A SHORT SYNTHESIS OF 4-HYDROXYPYRROLIDINE-2-ONE FROM TETRAMIC ACID INTERMEDIATES

Sharifah Nurin Zafirah[1], Noor Hidayah Pungot[1,2*], and Zurina Shaameri[1,2]

[1]Faculty of Applied Sciences, School of Chemistry and Environment, Universiti Teknologi MARA (UiTM), 40450 Shah Alam, Selangor, Malaysia
[2]Organic Synthesis Laboratory, Institute of Science, Universiti Teknologi MARA (UiTM), Puncak Alam Campus, 43200 Bandar Puncak Alam, Selangor, Malaysia

**Corresponding author: noorhidayah977@uitm.edu.my*

Abstract

4-Hydroxypyrrolidine-2-one **1** is useful as intermediates for synthesising antibiotic and antidepressant agents. Nevertheless, most organic synthesis routes are long and require a long time to generate the desired compounds, consisting of two reactions steps. The first step involves synthesising pyrrolidine-2,4-diones from N-Boc-amino acids via Meldrum's acid-mediated reaction and tetramic acid cyclisation. Generally, 1-(3-diemthylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC.HCl) is employed as a carboxyl activating agent in the reaction due to easy access and cost. In the present study, the short synthesis of 4-hydroxy pyrrolidin-2-one utilising N-Boc-alanine **2** and N-Boc-valine **3** as the amino acid was determined to produce tetramic acids **4** and **5**. The present study employed easily obtainable starting chemical compounds and was less costly to synthesise the 5-substituted pyrrolidine-2,4-diones via Meldrum's acid-mediated reaction and tetramic acid cyclisation. The amino acids employed were also readily protected by the tert-butoxycarbonyl (Boc) group to prevent the acids from reacting with other compounds. Resultantly, compounds **4** and **5** were successfully synthesised with 22% and 10% yields, respectively. The tetramic acids were then subjected to regioselective reduction with sodium borohydride ($NaBH_4$) as the reducing agent in methanol to acquire compounds **6** (9%) and **7** (6%). All synthesised compounds were purified by column chromatography and characterised using nuclear magnetic resonance (^1H and ^{13}C NMR) spectroscopy.

Keywords: pyrrolidine-2,4-dione, tetramic acid, Meldrum's acid, sodium borohydride, NMR spectroscopy.

Article History:- Received: 30 August 2021; Accepted: 30 March 2022; Published: 30 April 2022

Introduction

Pyrrolidine-2,4-dione **10**, commonly known as tetramic acid, is a crucial structural unit in numerous natural products and exists with dramatically varying structural complexities. Natural and synthetic tetramic acids have attracted the interest of biologists and chemists due to their wide range of biological characteristics, such as antibacterial, cytotoxicity, mycotoxicity, antiviral, anti-ulcerative, and tumour inhibitors in humans (Royles, 1995). Tetramic acid can be derived mostly from bacteria, fungi and various sponges and also from different terrestrial and marine species (Mo *et al.*, 2014). Both natural and synthetic tetramic acids have attracted the interests of many biologists and chemists. Furthermore, significant fractions of organic compounds containing nitrogen heterocycles are isolated from nature, including nicotine, vinblastine, and tryptamine, which possess biochemical, pharmaceutical, and agricultural values (Hamzah *et al*., 2013).

Recently, the synthesis of natural and synthetic pyrrolidine and pyrrolidinone derivatives has garnered scholarly attention due to their diverse biological characteristics. A considerable range of pyrrolidine alkaloids has been synthesised by employing structurally distinct 2-pyrrolidinones, which could be utilised as conventional synthetic subunits or chiral versions of biologically active molecules (Park *et al*., 2003). Moreover, 4-hydroxy-2-pyrrolidinone **1** and its *N*-substituted derivatives intermediates are

useful and could be easily and highly selectively produced as a raw material for producing drugs. The compounds are also beneficial for preparing other pharmaceuticals, including intermediates for synthesising antibiotics, such as oral antibiotic carbapenem that utilises the (S)-enantiomers, and antidepressant agents by employing the (R)-enantiomers (Chang *et al*., 2000).

Dieckmann condensation is a traditional method based on the transformation of carbonyl compounds and the new anionic type of enolate rearrangements that could be employed to synthesise tetramic acid (Tikhov & Kuznetsov, 2020). The reduction of carbonyls to alcohols in synthetic organic chemistry is an important transformation. Generally, a strong reducing agent, such as lithium aluminium hydride ($LiAlH_4$) is used. Although this reagent is very effective in the reduction process, it is extremely sensitive to moisture and cannot tolerate most reducible groups present in multifunctional molecules. Sodium borohydride ($NaBH_4$) will be used in this study for its attractiveness, unlike other hydrides, as it is cost-effective and less susceptible to moisture (Prasanth et al., 2018). The most popular and versatile amine protection groups used in all branches of chemistry are carbamates with benzyloxycarbonyl (Cbz), t-butoxycarbonyl (Boc), allyloxycarbonyl (Aloc or Alloc) and 9-fluorenylmethoxycarbonyl (Fmoc) substituents, as they provide robustness, ease of introduction and removal and opportunities for orthogonal deprotection (Lizza *et al.*, 2018). The tert-butoxycarbonyl (Boc) group has been commonly used to mask the amino feature because of the great stability against catalytic hydrogenation and resistance to basic conditions and many other nucleophilic reagents, finding widespread use in both organic and peptide synthesis, and eliminating the Boc protective group can be easily performed (Jahani *et al.*, 2011). Nevertheless, most organic synthesis routes are long and require a long time to generate the desired compounds. Consequently, the present study attempted to discover the most efficient, shorter, and high yield synthetic route to synthesise pyrrolidine-2,4-dione (**10**) by utilising easily obtainable and cheaper starting materials.

Methods

General procedures

All synthesised compounds were characterised using ^{1}H NMR spectroscopy and were dissolved in chloroform ($CDCl_3$), a deuterated solvent. The JEOL Resonance ECZ400S spectrometer was used to record the proton nuclear magnetic resonance spectra at 400 MHz.

Cyclisation of *N*-Boc-Alanine, 2 with Meldrum's acid to produce *tert*-butyl 2-methyl-3,5-dioxopyrrolidine-1-carboxylate, 4.

N-Boc-Alanine **2** (1.24 g, 13.88 mmol) and EDC.HCl (3.46 g, 18.04 mmol) were added to a solution of Meldrum's acid (2 g, 13.88 mmol) and DMAP (2.2 g, 18.04 mmol) in dichloromethane (40 mL) at 0°C. The mixture was stirred overnight at room temperature and then poured into ethyl acetate (200 mL). Then, the mixture was washed with brine (2 x 100 mL), 5% citric acid (3 x 300 mL) and again with brine (300 mL). The organic phase was then refluxed for 30 minutes and evaporated. TLC was used to monitor the reaction after 30 minutes, resulting two spots: one from the starting material, Meldrum's acid, and the other, which was considered to be the product. Thus, the impurified product undergo the purification process by column chromatography (ethyl acetate/methanol) to extract the desired product. When the purification process has completed, the solvent was evaporated using rotary evaporator to give *tert*-butyl 2-methyl-3,5-dioxopyrrolidine-1-carboxylate **4** in yellow crystallized form (0.64g, 22%). The analytical data of compound **4:** ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 5.03 (s, 2H), 4.40-4.37 (m, 1H), 3.22-3.20 (m, 3H), 1.53 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$): 12.5 (CH_3), 28.4 (3 x CH_3), 82.0 (C-O), 149.0 (C=O), 44.0 (CH_2), 59.0 (CH), 180.0 (C=O), 207.5 (C=O). Elemental analysis for $C_{10}H_{15}NO_4$: C, 56.33; H, 7.09; N, 6.57; O, 30.01.

Cyclisation of *N*-Boc-Valine, 3 with Meldrum's acid to produce *tert*-butyl 2-isopropylyl-3,5-dioxopyrrolidine-1-carboxylate, 5.

N-Boc-Valine **3** (1.63 g, 13.88 mmol) and EDC.HCl (3.46 g, 18.04 mmol) were added to a solution of Meldrum's acid (2 g, 13.88 mmol) and DMAP (2.2 g, 18.04 mmol) in dichloromethane (40 mL) at 0°C.

The mixture was stirred overnight at room temperature and then poured into ethyl acetate (200 mL). Then, the mixture was washed with brine (2 x 100 mL), 5% citric acid (3 x 300 mL) and again with brine (300 mL). The organic phase was refluxed for 30 minutes and evaporated. TLC was used to monitor the reaction after 30 minutes, and it revealed two spots: one from the starting material, Meldrum's acid, and the other, which was considered to be the product. Thus, the impurified product undergo the purification process by column chromatography (ethyl acetate/methanol) to extract the desired product. When the purification process has completed, the solvent was evaporated using rotary evaporator to give *tert*-butyl 2-isopropylyl-3,5-dioxopyrrolidine-1-carboxylate **5** in yellow crystallized form (0.33 g, 10%). The analytical data of compound **5**: ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 4.27 (d, *J* = 3.9 Hz, 1H), 3.10 (s, 2H), 2.35 (td, *J* = 7.1, 3.8 Hz, 1H), 1.54 (s, 9H), 1.11 (d, *J* = 6.9 Hz, 3H), 0.91 (d, *J* = 5.0 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): 19.0 (2 x CH_3), 25.5 (CH), 28.4 (3 x CH_3), 82 (C-O), 149 (C=O), 44 (CH_2), 59 (CH), 180 (C=O), 207.5 (C=O). Elemental analysis for $C_{12}H_{19}NO_4$: C, 59.73; H, 7.94; N, 5.81; O, 26.52.

Regioselective Reduction of *tert*-Butyl 2-methyl-3,5-dioxopyrrolidine-1-carboxylate, 4 with $NaBH_4$ to produce *tert*-Butyl 3-hydroxy-2-methyl-5-oxopyrrolidine-1-carboxylate, 6.

$NaBH_4$ (0.136 g, 3.60 mmol) was added to a stirred solution of *tert*-butyl 2-methyl-3,5-dioxopyrrolidine-1-carboxylate **4** (0.60 g, 3.00 mmol) in methanol (10 mL). The reaction mixture will be stirred at 0°C for 1 hour and at room temperature for 24 hours. After completion of the reaction, the solvent was removed via rotary evaporator. The residue will be partitioned between ethyl acetate and distilled water. The organic phase was collected, dried with anhydrous $MgSO_4$ and evaporated. The TLC was conducted resulted in several spots and shows prominent spots of *N*-Boc pyrrolidine-2,4-dione **4**. The crude product was purified using column chromatography (ethyl acetate/petroleum ether). After purification process, the solvent was evaporated via rotary evaporator giving the *tert*-butyl 3-hydroxy-2-methyl-5-oxopyrrolidine-1-carboxylate **6** in yellow residue (0.07 g, 9%). The analytical data of compound **6**: ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 4.45 (td, *J* = 8.0, 6.9 Hz, 1H), 4.21 (t, *J* = 6.6 Hz, 1H), 2.66 (dd, *J* = 17.2, 7.5 Hz, 1H), 2.55 (q, *J* = 8.7 Hz, 1H), 1.49 (s, 9H), 1.29 (d, *J* = 6.4 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): 14.5 (CH_3), 28.4 (3 x CH_3), 82.0 (C-O), 149.0 (C=O), 44.0 (CH_2), 59.0 (CH), 70.0 (C-OH), 176.5 (C=O). Elemental analysis for $C_{10}H_{17}NO_4$: C, 55.80; H, 7.96; N, 6.51; O, 29.73.

Regioselective Reduction of *tert*-Butyl 2-isopropyl-3,5-dioxopyrrolidine-1-carboxylate, 5 with $NaBH_4$ to produce *tert*-Butyl 3-hydroxy-2-isopropyl-5-oxopyrrolidine-1-carboxylate, 7.

$NaBH_4$ (0.06 g, 1.64 mmol) was added to a stirred solution of *tert*-butyl 2-isopropyl-3,5-dioxopyrrolidine-1-carboxylate, **5** (0.33 g, 1.37 mmol) in methanol (4 mL). The reaction mixture will be stirred at 0°C for 1 hour and at room temperature for 24 hours. After completion of the reaction, the solvent was removed via rotary evaporator. The residue will be partitioned between ethyl acetate and distilled water. The organic phase was collected, dried with anhydrous $MgSO_4$ and evaporated. The TLC was conducted resulted in several spots and shows prominent spots of *N*-Boc pyrrolidine-2,4-dione **5**. The crude product was purified using column chromatography (ethyl acetate/petroleum ether). After purification process, the solvent was evaporated via rotary evaporator giving the *tert*-butyl 3-hydroxy-2-isopropyl-5-oxopyrrolidine-1-carboxylate, **7** in yellow residue (0.02 g, 6%). The analytical data of compound **6**: ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 1.05 (d, 2H), 2.40 (s, 1H), 4.45 (td, *J* = 8.0, 6.9 Hz, 1H), 4.21 (t, *J* = 6.6 Hz, 1H), 2.66 (dd, *J* = 17.2, 7.5 Hz, 1H), 2.55 (q, *J* = 8.7 Hz, 1H), 1.49 (s, 9H), 1.29 (d, *J* = 6.4 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): 19.0 (2 x CH_3), 25.5 (CH), 28.4 (3 x CH_3), 82 (C-O), 149 (C=O), 44 (CH_2), 60.1 (CH), 69.5 (C-OH), 180 (C=O). Elemental analysis for $C_{12}H_{21}NO_4$: C, 59.24; H, 8.70; N, 5.76; O, 26.30.

Removal of N-Boc Protecting Group from *tert*-Butyl 3-hydroxy-2-methyl-5-oxopyrrolidine-1-carboxylate, 6 with TFA to produce 4-Hydroxy-5-methylpyrrolidin-2-one, 8.

tert-Butyl 3-hydroxy-2-methyl-5-oxopyrrolidine-1-carboxylate **6** (70 mg, 0.33 mmol) was dissolved in dichloromethane (1 mL). Trifluoroacetic acid (0.60 mL, 5.28 mmol, 16 equiv.) was added. The reaction

mixture was stirred at 25°C for 3 hours. Dichloromethane was evaporated off and replaced by anhydrous toluene which was then evaporated to azeotrope using excess trifluoroacetic acid. This procedure was repeated three times to yield an oil which was dried in vacuum. Anticipated product which was 4-hydroxy-5-methylpyrrolidin-2-one **8** can be obtained as a yellow oil in quantitative yield (Delsuc *et al*., 2007). Elemental analysis for $C_5H_9NO_2$: C, 52.16; H, 7.88; N, 12.17; O, 27.79.

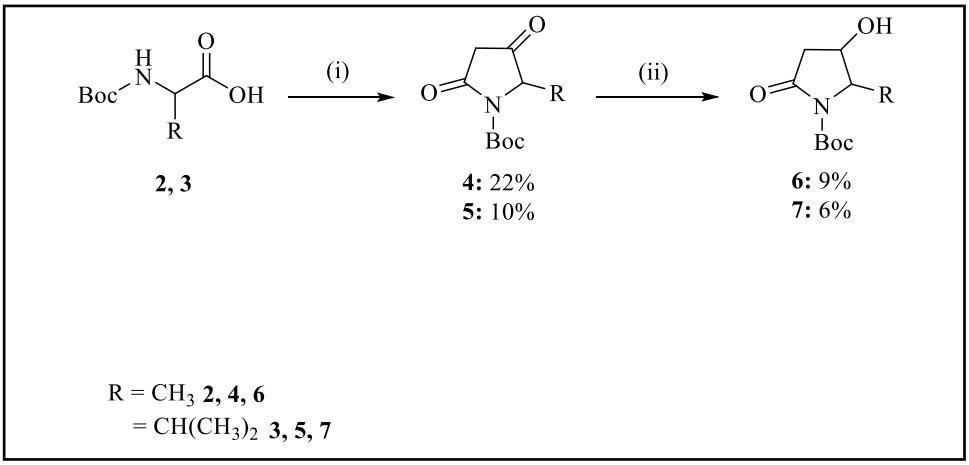
Removal of N-Boc Protecting Group from *tert*-Butyl 3-hydroxy-2-isopropyl-5-oxopyrrolidine-1-carboxylate, 7 with TFA to produce 4-Hydroxy-5-isopropylpyrrolidin-2-one, 9.

tert-Butyl 3-hydroxy-2-isopropyl-5-oxopyrrolidine-1-carboxylate **7** (20 mg, 0.08 mmol) was dissolved in dichloromethane. Trifluoroacetic acid (0.15mL, 1.28 mmol, 16 equiv.) was added. The reaction mixture was stirred at 25°C for 3 hours. Dichloromethane was evaporated off and replaced by anhydrous toluene which was then evaporated to azeotrope using excess trifluoroacetic acid. This procedure was repeated three times to yield an oil which was dried in vacuum. Anticipated product which is 4-hydroxy-5-isopropylpyrrolidin-2-one **9** can be obtained as a yellow oil in quantitative yield (Delsuc *et al*., 2007). Elemental analysis for $C_7H_{13}NO_2$: C, 58.72; H, 9.15; N, 9.78; O, 22.35.

Results and Discussion

In the preparation of tetramic acid, the readily available Meldrum's acid was employed as the starting material to prepare tetramic acid. Based on Scheme 1, the protected amino acids, *N*-Boc-alanine **2** and *N*-Boc-valine **3** underwent cyclisation to yield the tetramic acids. The base utilised in the reaction was DMAP, which acted as the nucleophilic catalyst. Additionally, EDC.HCl was the carboxyl activating agent employed to activate the oxygen on the carboxylate group into a reactive acylating group, thus forming an intermediate consisting of a good leaving group as the side product known as urea. The tetramic acids **4** and **5** were obtained in low yields of 22% and 10%, respectively.

Carbonyl compounds could produce alcohols in the regioselective reduction of tetramic acid with hydride reductions while adding two hydrogen atoms to the C=O bonds triggers the reductions (Delsuc *et al*., 2007). The keto functionality was regioselectively reduced with $NaBH_4$, the reducing agent, in methanol to furnish its hydroxyl functionality, yielding 9% of **6** and 6% of **7**. One of the most common amino protecting groups is the *tert*-butoxycarbonyl (Boc) group. The deprotection reaction of Boc group was reviewed based on the procedures reported by Delsuc and co-workers, which manufactured the targeted products **8** and **9** in quantitative yield.



Scheme 1. Overall synthetic route of short synthesis of 4-Hydroxy pyrrolidin-2-one from tetramic acid. Conditions: (i) Meldrum's acid, DMAP, EDC.HCl, overnight, rt (ii) $NaBH_4$, methanol, 24h, rt

Conclusion

In the current study, tetramic acid cyclisation and Meldrum's acid-mediated reaction to synthesise 5-substituted pyrrolidine-2,4-diones, compounds **4** and **5**, in the presence of DMAP, EDC.HCL in dichloromethane was successful at 22% and 10% yields, respectively. N-Boc-alanine **2** and N-Boc-valine **3** were employed as the N-Boc protected amino acids during the reaction. Subsequently, the 5-substituted pyrrolidine-2,4-diones were regioselectively reduced in NaBH4 as the reducing agent in methanol. Consequently, 4-hydroxy pyrrolidin-2-one, denoted as compounds **6** and **7**, were acquired at 9% and 6%, respectively. All of the synthesised compounds were characterised by ^{1}H and ^{13}C NMR spectroscopy. The present research is beneficial to the scientific community, especially chemists and pharmacists, as the discovered shorter synthetic route for 4-hydroxypyrrolidin-2-one could significantly contribute to the pharmaceutical and chemical industries.

Acknowledgement

The authors acknowledge the generous support of Organic Synthesis Laboratory, Institute of Science (IOS), Universiti Teknologi Mara (UiTM) in providing required chemicals and instrumentations, as well as the financial support of Ministry of Higher Education Malaysia (FRGS grant 600-IRMI/FRGS 5/3 (399/2019).

References

Chang, D., Witholt, B., & Li, Z. (2000). Preparation of (S)-N-Substituted 4-Hydroxy-pyrrolidin-2-ones by Regio- and Stereoselective Hydroxylation with Sphingomonas sp. HXN-200. *Organic Letters*, *2*(24), 3949–3952. https://doi.org/10.1021/ol006735q

Delsuc, N., Léger, J. M., Massip, S., & Huc, I. (2007). Proteomorphous objects from abiotic backbones. *Angewandte Chemie - International Edition*, *46*(1–2), 214–217. https://doi.org/10.1002/anie.200603390

Hamzah, A. S., Shaameri, Z., & Goksu, S. (2013). Five-membered nitrogen heterocyclic compounds. *Journal of Chemistry*, *2013*. https://doi.org/10.1155/2013/250381

Jahani, F., Tajbakhsh, M., Golchoubian, H., & Khaksar, S. (2011). Guanidine hydrochloride as an organocatalyst for N-Boc protection of amino groups. *Tetrahedron Letters*, *52*(12), 1260–1264. https://doi.org/10.1016/j.tetlet.2011.01.023

Lizza, J. R., Bremerich, M., McCabe, S. R., & Wipf, P. (2018). 2,2,6,6-Tetramethylpiperidin-1-yloxycarbonyl: A Protecting Group for Primary, Secondary, and Heterocyclic Amines. *Organic Letters*, *20*(21), 6760–6764. https://doi.org/10.1021/acs.orglett.8b02874

Mo, X., Li, Q., & Ju, J. (2014). Naturally occurring tetramic acid products: Isolation, structure elucidation and biological activity. *RSC Advances*, *4*(92), 50566–50593. https://doi.org/10.1039/c4ra09047k

Park, T. H., Paik, S., & Lee, S. H. (2003). A Practical Synthesis of (S)- and (R)-4-Hydroxy-2-pyrrolidinone via 1-Phenylethylamine Mediated Resolution. *Bulletin of the Korean Chemical Society*, *24*(8), 1227–1228. https://doi.org/10.5012/bkcs.2003.24.8.1227

Prasanth, C. P., Joseph, E., Abhijith, A., Nair, D. S., Ibnusaud, I., Raskatov, J., & Singaram, B. (2018). Stabilization of NaBH4 in Methanol Using a Catalytic Amount of NaOMe. Reduction of Esters and Lactones at Room Temperature without Solvent-Induced Loss of Hydride. *Journal of Organic Chemistry*, *83*(3), 1431–1440. https://doi.org/10.1021/acs.joc.7b02993

Royles, B. J. L. (1995). Naturally Occurring Tetramic Acids: Structure, Isolation, and Synthesis. *Chemical Reviews*, *95*(6), 1981–2001. https://doi.org/10.1021/cr00038a009

Tikhov, R. M., & Kuznetsov, N. Y. (2020). Construction of piperidine-2,4-dione-type azaheterocycles and their application in modern drug development and natural product synthesis. Organic and Biomolecular Chemistry, 18(15), 2793–2812. https://doi.org/10.1039/d0ob00287a