The Malaysian Journal of ANALYTICAL SCIENCES ISSN 1394-2506

CHEMICAL EXPLORATION OF 4-HYDROXYBENZYLATED 3-SUBSTITUTED TETRAMIC ACID

(Penerokaan Kimia bagi 4-Hidroksibenzil 3-Gantian Asid Tetramik)

Norhanim Mohalid[1], Ahmad Sazali Hamzah[2], Zurina Shaameri[2*]

[1]Faculty of Applied Sciences,
[2]Organic Synthesis Laboratory, Institute of Science,
Universiti Teknologi MARA (UiTM), 40450 Shah Alam, Selangor, Malaysia

**Corresponding author: zurina@salam.uitm.edu.my*

Received: 26 February 2014; Accepted: 27 January 2015

Abstract

The tetramic acid (pyrrolidine-2,4-dione) ring system was discovered as a key structural unit in many natural products. The tetramic acid moiety, in most cases, is present as a 3-acyl derivative. Fuligorubin A, a yellow pigment produced from *Fuligo septica*, consists of a hydroxybenzyl substituent at the C-5 position of the tetramic acid ring has been reported to show antibiotic and cytotoxic activities. In this project, a derivative of Fuligorubin A, namely 4-hydroxybenzylated 3-acyl tetramic acid from slime mold *Leocarpus fragilis* is chosen as the synthetic target not only due to the strong biological activity of Fuligorubin A, but mainly due to the challenging structure of the hydroxybenzylated tetramic acid moiety. We are also interested in synthesizing 3,4-fused ring (bicyclic) compounds because they might have comparable medicinal properties with the bioactive compounds, and tetramic acid is the key structural unit. Such fused ring system could be the lead synthon towards the synthesis of lactacystin derivatives. In the development towards the synthesis of the target compounds, our research group has attempted to synthesize them using *L*-tyrosine as the starting material. All synthesized compounds were characterized using Nuclear Magnetic Resonance (NMR) and Fourier Transform Infrared (FTIR) spectroscopy.

Keywords: pyrrolidine-2,4-dione, tetramic acid, acyltetramic acid, fused ring, bicyclic

Abstrak

Sistem cecincin asid tetramik (pirolidin-2,4-dion) telah ditemui sebagai unit struktur utama dalam kebanyakan hasilan semulajadi. Pada bahagian asid tetramik, dalam kebanyakan kes, hadir sebagai terbitan 3-asil. Fuligorubin A, pigment kuning yang dihasilkan dari *Fuligo septica*, mengandungi gantian hidroksibenzil di kedudukan C-5 pada cecincin asid tetramik telah dilaporkan menunjukkan aktiviti antibiotik dan sitotoksik. Dalam projek ini, satu terbitan dari Fuligorubin A, yang dinamakan 4-hidroksibenzil 3-asil asid tetramik dari lendir kulat *Leocarpus fragilis* telah dipilih sebagai sasaran sintesis bukan sahaja disebabkan aktiviti biologi Fuligorubin A yang kuat, tetapi terutamanya disebabkan oleh struktur hidroksibenzil pada bahagian asid tetramik yang mencabar. Kami juga berminat untuk menghasilkan sebatian 3,4-cecincin bersatu (bisiklik) kerana ia mungkin mempunyai ciri-ciri perubatan yang sama dengan sebatian bioaktif, dan asid tetramik adalah unit struktur utamanya. Sistem cecincin bersatu tersebut boleh menjadi sinton utama terhadap penghasilan terbitan lactacystin. Dalam perkembangan terhadap penghasilan sebatian sasaran, kumpulan penyelidikan kami telah mencuba untuk menghasilkannya menggunakan *L*-tirosina sebagai bahan permulaan. Kesemua sebatian yang dihasilkan telah dicirikan menggunakan spektroskopik *Nuclear Magnetic Resonance* (NMR) dan *Fourier Transform Infrared* (FTIR).

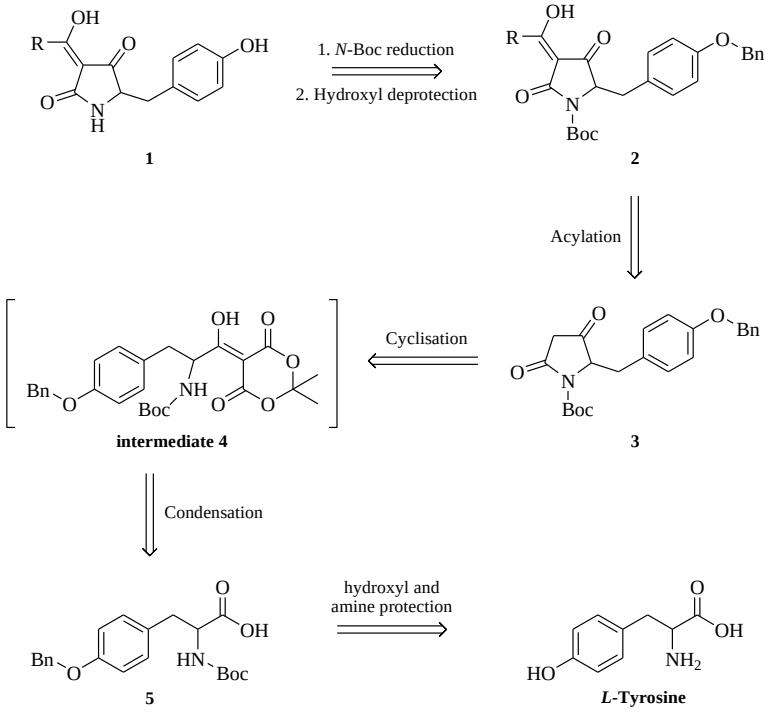
Kata kunci: pirolidin-2,4-dion, asid tetramik, asid asiltetramik, gabungan cecincin, bisiklik

Introduction

Tetramic acid, or pyrrolidine-2,4-dione, is an important class of naturally occurring molecules. It has been known since the early twentieth century. However, its importance was realized in 1960s when it was discovered as a key

structural unit in many natural products such as antiprotozoal malonomycin, terminal deoxyribonucleic acid (DNA) transferase inhibitor streptolydigin and antibiotic tirandamycin. The spectrum of biological activity displayed by these tetramic acid-containing natural products is extremely diversified which include antibiotic, antiviral and antiulcerative properties, cytotoxicity and mycotoxicity, tumor inhibition as well as fungicidal action. Synthetic analogues of certain tetramic acids have also been reported to be subjects of clinical investigation, predominantly as antibiotic [1].

Our first main concern is to develop a flexible, novel and simple synthetic route towards the synthesis of an important tetramic acid skeleton, *via* Meldrum acid's mediated reaction and tetramic acid cyclisation. The tetramic acid derivatives that most commonly found in nature are pyrrolidine-2,4-dione containing an acyl substitution at C-3 position. Fuligorubin A, isolated by Steglich and co-workers (1987) from *Fuligo septica*, is a yellow-coloured plasmodial pigment containing polyenoyltetramic acid [2]. Apart from that, 4-hydroxybenzylated 3-acetyltetramic acid **1** is one of a series of orange-yellow colour of plasmodia in the slime mould *Leocarpus fragilis* isolated by Steglich (1989). It was found together with three other compounds, which are biogenetically derived from *L*-tyrosine [3]. The retrosynthetic analysis of 4-hydroxybenzylated 3-acyltetramic acid, which comprises of five steps, is outlined in Scheme 1 below:



Scheme 1: Retrosynthetic Analysis of 4-Hydroxybenzylated 3-Acyltetramic Acid

After successfully synthesizing the acylated tetramic acid **1**, we decided to expand our study by synthesizing the derivatives that could lead to novel heterocyclic compounds with a pyrrolidine ring system. The tetramic acid **3** was envisioned to undergo alkylation at C-3 position followed by reduction and intramolecular cyclisation to produce new 3,4-fused γ-lactam-γ-lactone and 3,4-fused γ-lactam-γ-lactam bicyclic core. Such fused ring systems might have comparable medicinal properties with bioactive compounds like neooxazolomycin, salinosporamide A, omuralide and cinnabaramide (Figure 1) [4]. 3,4-Fused ring system has never been synthesized nor explored as a potential synthon, yet it has the potential to be an advanced intermediate towards the synthesis of analogues of lactacystin series of lactams.

Neooxazolomycin, isolated from the fermentation broth of Streptomyces has received much attention as a candidate for antibiotic and anticancer drugs [5]. However, bearing a γ-lactone ring as a part of its structural conformation fused to a γ-lactam, the link is at 4,5-position and not at 3,4-position as investigated in this work. In addition, the synthesis of 3,4-fused γ-lactam-γ-lactam is never attempted. By referring to the synthesis of tetramic acid skeleton as outlined in Scheme 1 above, additional sequence of reactions can lead to the formation of a different ring system which can be a multifunctional moiety to many new polysubstitutional fused ring systems (Scheme 2).

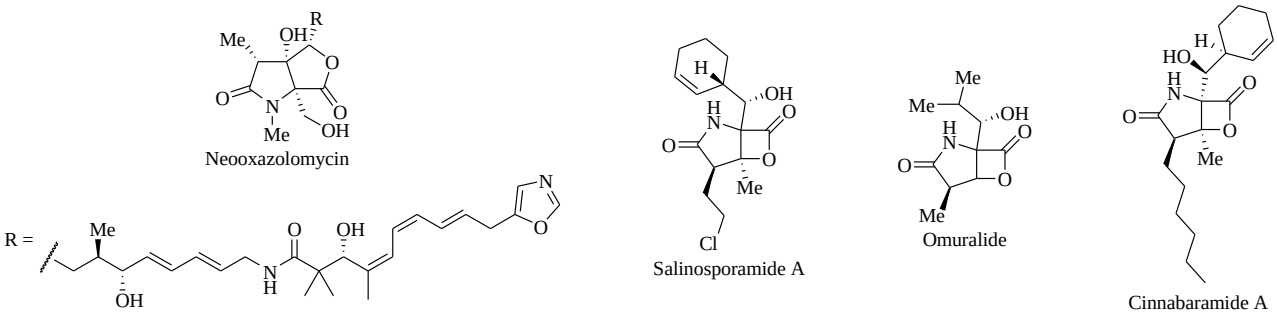
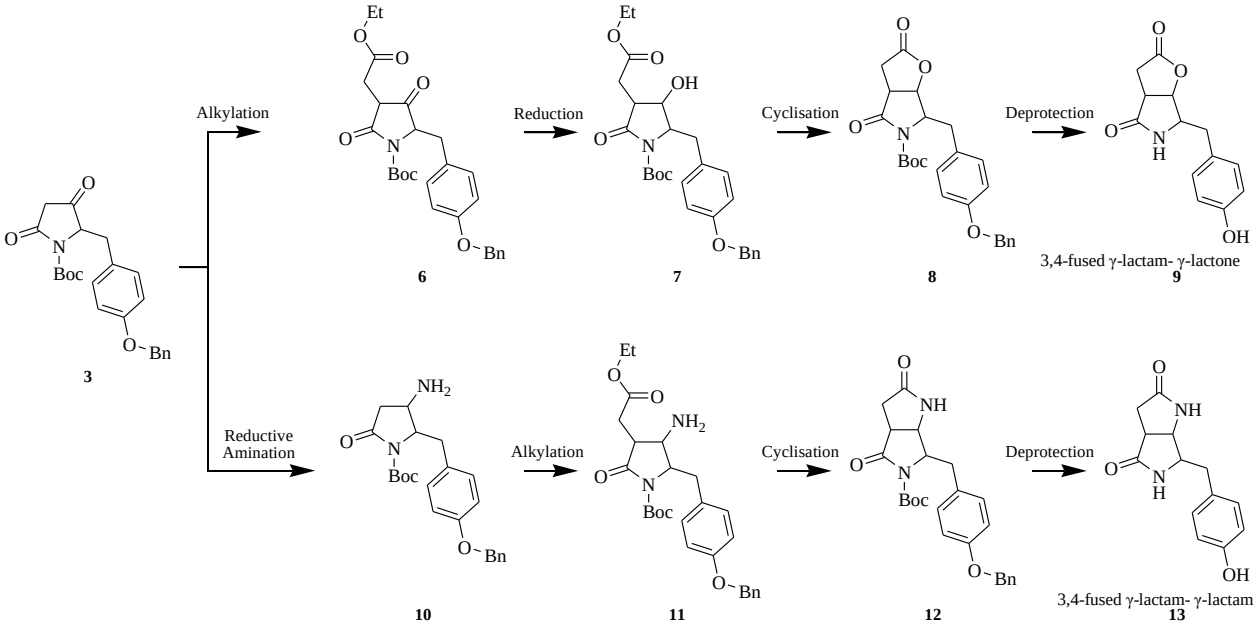


Figure 1. Molecular Structures of Some Bioactive Compounds with Fused Ring Systems



Scheme 2. Proposed Synthesis of 3,4-Fused Ring (Bicyclic) Compounds

Materials and Methods

General Procedure

^{1}H- and ^{13}C-NMR spectra were measured at 300 and 75 MHz, respectively. All NMR spectra were recorded in deuterated solvents on Varian NMR 300 MHz or Bruker NMR 300 MHz Spectrometers with tetramethylsilane as an internal standard. Chemical shifts are expressed in δ (ppm) units downfield from TMS. Infrared spectra were measured on Varian Excalibur 3100. All samples were run neat on a single refraction ZnSe crystal plate *via* ATR sampling accessory. Elemental analyses were performed on Flash EA 110 instrument. Melting points were determined by either an Electrothermal melting point apparatus or an automatic B-545 melting point apparatus from Büchi and were uncorrected.

2-Amino-3-[4-(benzyloxy)phenyl]propanoic acid, 14.

To a solution of *L*-tyrosine (3.62 g, 20 mmol) in 2 M NaOH (10 mL, 20 mmol), a solution of copper(II) sulfate pentahydrate ($CuSO_4.5H_2O$) (2.50 g, 10 mmol) in water (10 mL) was added under stirring at room temperature. After one hour of reflux, the mixture was allowed to cool to room temperature, and was dissolved in methanol (MeOH) (75 mL) and 2 M NaOH (10 mL, 20 mmol). Benzyl bromide (2.5 mL, 21 mmol) was added, and the mixture was stirred at room temperature overnight. The precipitate was collected, washed with water and methanol, transferred to an Erlenmeyer flask and stirred with 1 M HCl (40 mL) for one hour. The precipitate was filtered, washed with water and treated with 1 M ammonia (NH_3) (2 x 40 mL), again washed with water (50 mL) and acetone (24 mL), and dried to give the benzylated *L*-tyrosine **6** as white powder (3.85 g, 71%), m.p 266.3°C. IR *v* cm^{-1}: 3297 (N-H), 1718 (C=O); CHN: Found C, 71.20; H, 6.07; N, 6.36; O, 16.37 %; requires C, 70.83; H, 6.32; N, 5.16; O, 17.69 %.

3-[4-(Benzyloxy)phenyl]-2-(*tert*-butoxycarbonylamino)propanoic acid, 5

To the crude benzylated L-tyrosine **14** (9.50 g, 35 mmol) suspended in dioxane-water (2:1, 210 mL), 1 M NaOH (35.0 mL, 35 mmol) and sodium hydrogen carbonate ($NaHCO_3$) (2.94 g, 35 mmol) were added. To the reaction mixture cooled in ice-water, $(Boc)_2O$ (15.28 g, 70 mmol) was added and stirred at room temperature overnight. Undissolved material was discarded and the filtrate was partially evaporated (to approximately 180 mL). The aqueous residue was cooled in ice-water, ethyl acetate (120 mL) was added and the mixture was acidified to pH=2-3 by addition of 1 M potassium hydrogen sulfate ($KHSO_4$) (approximately 60 mL). Layers were separated; the aqueous layer was extracted with ethyl acetate (2 x 36 mL), and the combined extracts were washed with water, dried over anhydrous magnesium sulfate ($MgSO_4$), and evaporated to give the Boc-protected L-tyrosine **5** as yellowish thick oil (9.75 g, 75%). IR *v* cm^{-1}: 3308 (N-H), 1749 (C=O), 1713 (C=O); δ_H ($CDCl_3$, 300 MHz): 1.42 (9H, s, $(CH_3)_3$), 3.04 (1H, dd, J = 6 Hz and J = 6 Hz, NCHCH*H*), 3.13 (1H, dd, J = 6 Hz and J = 6 Hz, NCHC*H*H), 4.58 (1H, q, J = 6, 6 and 6 Hz, NCH), 4.97 (1H, d, J = 9 Hz, NH), 5.03 (2H, s, OCH_2), 6.92 (2H, d, J = 9 Hz, Ar H), 7.11 (2H, d, J = 9 Hz, Ar H), 7.31-7.43 (5H, m, Ar H), 7.68 (1H, br s, OH); ^{13}C ($CDCl_3$, 75 MHz): 28.3 (CH_3), 36.9 (CH_2), 54.4 (NCH), 70.0 (OCH_2), 80.3 (quat. C), 114.9 (Ar C), 127.5-128.6 (Ar C), 130.4 (quat. Ar C), 137.0 (quat. C), 155.4 (COOR), 157.9 (quat. C), 176.5 (COOH); CHN: Found C, 68.63; H, 6.27; N, 4.66; O, 20.44 %; requires C, 67.91; H, 6.78; N, 3.77; O, 21.54 %.

***tert*-Butyl 2-[4-(benzyloxy)benzyl]-3,5-dioxopyrrolidine-1-carboxylate, 3**

To a solution of Meldrum's acid (1.52 g, 10.54 mmol) and DMAP (1.64 g, 13.41 mmol) in dichloromethane (60 mL) at 0°C was added 3-(4-(benzyloxy)phenyl)-2-(*tert*-butoxy-carbonylamino)propanoic acid **5** (3.56 g, 9.58 mmol) and then EDC.HCl (2.20 g, 11.50 mmol). The mixture was stirred overnight at room temperature and poured into ethyl acetate (200 mL), washed with brine (2 x 100 mL), 5% citric acid (3 x 300 mL) and again brine (300 mL). The organic phase was refluxed for 30 min and evaporated, which then washed with diethyl ether to give white fluffy powder **3** (2.65 g, 70%), m.p. 162.2°C. IR *v* cm^{-1}: 1745 (C=O), 1714 (C=O), 1689 (C=O); δ_H ($CDCl_3$, 300 MHz): 1.62 (9H, s, $(CH_3)_3$), 2.28 (1H, d, J = 24 Hz , COCH*H*), 2.86 (1H, d, J = 24 Hz, COC*H*H), 3.16 (1H, dd, J = 3 Hz and J = 3 Hz, NCHCH*H*), 3.34 (1H, dd, J = 6 Hz and J = 6 Hz, NCHC*H*H), 4.61 (1H, m, NCH), 5.01 (2H, s, OCH_2), 6.88 (2H, d, J = 6 Hz, Ar H), 6.94 (2H, d, J = 9 Hz, Ar H), 7.32-7.40 (5H, m, Ar H); ^{13}C ($CDCl_3$, 75 MHz): 28.1 (CH_3), 35.7 (NCH*C*H_2), 43.4 (C=O*C*H_2), 68.4 (CH), 70.0 (OCH_2), 84.4 (quat. C), 115.3 (Ar C), 125.9 (quat. C), 127.6-130.9 (Ar C), 136.7 (quat. C), 149.1 (*C*OOC$(CH_3)_3$), 158.2 (quat. C), 167.6 (N*C*OCH_2), 204.5 (NCH*C*O); CHN: Found C, 70.82; H, 4.24; N, 4.02; O, 18.92 %; requires C, 68.91; H, 6.57; N, 3.65; O, 20.86 %.

***tert*-Butyl 3-benzoyl-5-[4-(benzyloxy)benzyl]-2,4-dioxopyrrolidine-1-carboxylate, 4b**

Potassium tert-butoxide activation - To a stirred solution of *tert*-butyl 2-(4-(benzyloxy)benzyl)-3,5-dioxopyrrolidine-1-carboxylate **3** (1.19 g, 3.0 mmol) in 45 mL of dry DMF under nitrogen was added potassium *tert*-butoxide (0.37 g, 3.3 mmol) under cooling at 0°C. After 30 minutes, benzoyl chloride (0.42 mL, 3.0 mmol) was added to the stirred solution. The mixture was stirred for two days at room temperature under nitrogen. The reaction mixture was treated with water, adjusted to pH 3 with diluted HCl and shaken with benzene. The benzene layer was washed with saturated brine, dried over anhydrous sodium sulfate (Na_2SO_4), and evaporated *in vacuo*. The crude product was subjected to column chromatography over silica gel (ethyl acetate/petroleum ether) to give **4b** as colourless crystal (0.55 g, 37%), IR *v* cm^{-1}: 1746 (C=O), 1714 (C=O), 1696 (C=O), 1700 (C=O); δ_H ($CDCl_3$, 300 MHz): 1.63 (9H, s, $C(CH_3)_3$), 3.34 (2H, m, $NCHCH_2$), 4.93 (1H, m, NCH), 4.96 (2H, s, OCH_2), 6.12 (1H, s, COCHCO), 6.82 (2H, d, J = 9 Hz, Ar H), 6.98 (2H, d, J = 9 Hz, Ar H), 7.31-7.43 (5H, m, Ar H), 7.45-7.94 (5H, m, Ar H); ^{13}C ($CDCl_3$, 75 MHz): 28.2 ($C(CH_3)_3$), 35.6 ($NCHCH_2$), 60.8 (NCH), 69.9 (COCHCO), 83.3 (quat. C), 108.2 (OCH_2), 114.9 (Ar C), 126.4 (quat. Ar C), 127.5 (Ar C), 128.0 (Ar C), 128.6 (Ar C), 128.9 (Ar C), 130.4 (Ar C), 134.7 (quat. Ar C), 136.9 (quat. Ar C), 149.2 (C=O), 158.0 (quat. Ar C), 161.8 (C=O), 165.5 (C=O), 168.2 (C=O)

Sodium hydride activation - To a stirred solution of *tert*-butyl 2-(4-(benzyloxy)benzyl)-3,5-dioxopyrrolidine-1-carboxylate **3** (1.19 g, 3 mmol) and 60% sodium hydride (0.12 g, 3 mmol) in 15 mL of dry DMF was added at -25°C under nitrogen, benzoyl chloride (0.42 mL, 3 mmol). The cooling bath was removed and temperature of the reaction mixture was allowed to rise. The mixture was continued to stir overnight at room temperature and then evaporated to dryness *in vacuo*. The resulting oil was treated with benzene and diluted HCl with shaking. The organic layer was washed with water, dried with Na_2SO_4 and evaporated to dryness. The crude product was subjected to column chromatography over silica gel (ethyl acetate/petroleum ether) to give **4b** as colourless crystal (0.37 g, 25%). The spectroscopic data of **4b** obtained *via* sodium hydride activation are the same as those obtained *via* potassium *tert*-butoxide activation.

(*E*)-*tert*-Butyl 2-[4-(benzyloxy)benzyl]-4-but-2-enoyl-3,5-dioxopyrrolidine-1-carboxylate, 4c

Potassium tert-butoxide activation – It was prepared as described for compound **4b** from *tert*-butyl 2-(4-(benzyloxy)benzyl)-3,5-dioxo-pyrrolidine-1-carboxylate **3** (1.19 g, 3.0 mmol) with 2-butenoyl chloride (0.29 mL, 3.0 mmol). The work up procedure was the same as that of **4b**. The crude product was subjected to column chromatography over silica gel (ethyl acetate/petroleum ether) to give **4c** as yellowish crystal (0.29 g, 21%), IR *v* cm^{-1}: 1749 (C=O), 1716 (C=O), 1691 (C=O), 1718 (C=O); δ_H ($CDCl_3$, 300 MHz): 1.62 (9H, s, $C(CH_3)_3$), 1.99 (3H, dd, J = 3 Hz and J = 3 Hz, CH=CHCH_3) 3.19 (1H, dd, J = 3 Hz and J = 3 Hz, NCHCH*H*), 3.33 (1H, dd, J = 6 Hz and J = 6 Hz, NCHC*H*H), 4.81 (1H, q, J = 3, 3 and 3 Hz, NCH), 5.01 (2H, s, OCH_2), 5.90 (1H, d, J = 15 Hz, COC*H*=CH), 5.97 (1H, s, COCHCO), 6.85 (2H, d, J = 9 Hz, Ar H), 6.93 (2H, d, J = 9 Hz, Ar H), 7.10 (1H, m, COCH=C*H*), 7.33-7.45 (5H, m, Ar H); ^{13}C ($CDCl_3$, 75 MHz): 18.6 (CH=CHCH_3), 28.2 ($C(CH_3)_3$), 34.9 ($NCHCH_2$), 60.7 (NCH), 69.9 (COCHCO), 83.2 (quat. C), 107.8 (OCH_2), 114.2 (Ar C), 120.4 (COCH=CH), 126.1 (quat. Ar C), 127.6 (Ar C), 128.0 (Ar C), 128.6 (Ar C), 130.5 (Ar C), 136.9 (quat. Ar C), 149.3 (CH_3CH=CH), 150.5 (C=O), 157.9 (quat. Ar C), 161.2 (C=O), 165.2 (C=O), 168.4 (C=O).

Sodium hydride activation - It was prepared as described for compound **4b** from *tert*-butyl 2-(4-(benzyloxy)benzyl)-3,5-dioxopyrrolidine-1-carboxylate **3** (1.19 g, 3.0 mmol) with 2-butenoyl chloride (0.29 mL, 3.0 mmol). The work up procedure was the same as that of **4b.** The crude product was subjected to colum chromatography over silica gel (ethyl acetate/petroleum ether) to give **4c** as yellowish crystal (0.28 g, 20%). The spectroscopic data of **4c** obtained *via* sodium hydride activation are the same as those obtained *via* potassium *tert*-butoxide activation.

***tert*-Butyl 2-(4-(benzyloxy)benzyl)-4-(2-ethoxy-2-oxoethyl)-3,5-dioxopyrrolidine-1-carboxyl-ate, 6**

To a stirred solution of *tert*-butyl 2-(4-(benzyloxy)benzyl)-3,5-dioxopyrrolidine-1-carboxylate **3** (1.19 g, 3.0 mmol) in THF (20 mL), TBAF.$3H_2O$ (1.42 g, 4.5 mmol) was added. The reaction mixture was stirred until all solid dissolved. Ethyl bromoacetate (0.4 mL, 3.6 mmol) was then added and the mixture was left stirring overnight. The reaction mixture was evaporated under reduced pressure at 40°C and the residue was chromatographed over silica gel (ethyl acetate/petroleum ether) to give **6** as yellowish oil (0.14 g, 10%), IR *v* cm^{-1}: 1744 (C=O), 1735 (C=O), 1716 (C=O), 1683 (C=O); δ_H ($CDCl_3$, 300 MHz): 1.28 (3H, t, CH_3), 1.57 (9H, s, $C(CH_3)_3$), 3.12 (1H, dd, J = 3 Hz

and J = 3 Hz, NCHC*H*H), 3.39 (1H, dd, J = 6 Hz and J = 6 Hz, NCHCH*H*), 4.25 (2H, m, $COOCH_2$), 4.45 (2H, dd, J = 3 Hz and J = 3 Hz, COCHCH_2), 4.71 (1H, m, NCH), 4.77 (1H, m, NCOCH), 4.97 (2H, s, OCH_2), 6.80 (2H, d, J = 9 Hz, Ar H), 6.95 (2H, d, J = 9 Hz, Ar H), 7.30-7.39 (5H, m, Ar H); ^{13}C ($CDCl_3$, 75 MHz): 14.1 (CH_3), 28.2 (C(CH_3)$_3$), 34.4 (NCHCH_2), 60.3 (COCHCH_2), 62.0 (NCO*C*H), 67.3 ($COOCH_2$), 69.8 (N*C*HCH_2), 82.8 (OCH_2), 96.5 (quat. C), 114.6 (Ar C), 126.2 (quat. Ar C), 127.6 (Ar C), 127.9 (Ar C), 128.6 (Ar C), 130.6 (Ar C), 136.9 (quat. Ar C), 149.2 (C=O), 157.8 (quat. Ar C), 166.3 (C=O), 168.4 (C=O), 174.3 (C=O).

***tert*-Butyl 2-(4-(benzyloxy)benzyl)-4-(2-ethoxy-2-oxoethyl)-3-hydroxy-5-oxopyrrolidine-1-carboxylate, 7**

To a cooled stirred solution of alkylated tetramic acid **6** (3.23 g, 6.70 mmol) in methanol (20 mL), $NaBH_4$ (0.30 g, 8.04 mmol) was added in portions over a period of 10 minutes. The mixture was stirred at 0°C for two hours and was then evaporated. The oily residue was partitioned between ethyl acetate and saturated ammonium chloride (NH_4Cl). The organic fraction was washed with water, dried over $MgSO_4$ and evaporated. The crude residue was subjected to flash chromatography over silica gel (ethyl acetate/methanol) to obtain product **7** as white solid (1.39 g, 43%), IR ν cm^{-1}: 1744 (C=O), 1735 (C=O), 1683 (C=O); δ_H ($CDCl_3$, 300 MHz): 1.29 (3H, m, CH_3), 1.64 (9H, s, C(CH_3)$_3$), 3.15 (1H, dd, J = 3 Hz and J = 3 Hz, NCHC*H*H), 3.46 (1H, dd, J = 6 Hz and J = 6 Hz, NCHCH*H*), 3.81 (1H, m, C*H*OH) 4.27 (2H, m, $COOCH_2$), 4.50 (2H, dd, J = 3 Hz and J = 3 Hz, COCHCH_2), 4.72 (1H, m, NCH), 4.79 (1H, m, NCOCH), 4.98 (2H, s, OCH_2), 6.87 (2H, d, J = 9 Hz, Ar H), 6.98 (2H, d, J = 9 Hz, Ar H), 7.37-7.46 (5H, m, Ar H); ^{13}C ($CDCl_3$, 75 MHz): 15.8 (CH_3), 29.9 (C(CH_3)$_3$), 36.9 (NCHCH_2), 61.5 (COCHCH_2), 63.7 (NCO*C*H), 65.2 (CHOH), 69.9 ($COOCH_2$), 70.3 (N*C*HCH_2), 83.0 (OCH_2), 97.2 (quat. C), 114.7 (Ar C), 126.9 (quat. Ar C), 127.9 (Ar C), 128.6 (Ar C), 129.0 (Ar C), 130.1 (Ar C), 136.0 (quat. Ar C), 151.2 (C=O), 151.5 (quat. Ar C), 169.0 (C=O), 170.9 (C=O).

(*Z*)-*tert*-Butyl 2-(4-(benzyloxy)benzyl)-3-(hydroxyimino)-5-oxopyrrolidine-1-carboxylate, 15

To a solution of *tert*-butyl 2-(4-(benzyloxy)benzyl)-3,5-dioxopyrrolidine-1-carboxylate **3** (4.15 g, 10.5 mmol) in dichloromethane (160 mL) were added $NH_2OH.HCl$ (1.31 g, 18.9 mmol), $NaHCO_3$ (1.59 g, 18.9 mmol) and Na_2SO_4 (2.39 g, 16.8 mmol). The mixture was stirred at room temperature for 48 hours, filtered and evaporated to furnish oxime **15** as yellowish thick oil (2.97 g, 69%), IR ν cm^{-1}: 3380 (O-H), 2243 (C=N), 1748 (C=O), 1669 (C=O); δ_H ($CDCl_3$, 300 MHz): 1.62 (9H, s, C(CH_3)$_3$), 2.28 (1H, d, J = 24 Hz, COC*H*H), 2.86 (1H, d, J = 24 Hz, COCH*H*), 3.16 (1H, dd, J = 3 Hz and J = 3 Hz, NCHC*H*H), 3.34 (1H, dd, J = 6 Hz and J = 6 Hz, NCHCH*H*), 4.61 (1H, m, NC*H*CH_2), 5.01 (2H, s, OCH_2), 6.88 (2H, d, J = 9 Hz, Ar H), 6.94 (2H, d, J = 9 Hz, Ar H), 7.30-7.43 (5H, m, Ar H); ^{13}C ($CDCl_3$, 75 MHz): 14.2 (NCH*C*H_2), 21.1 (CO*C*H_2), 28.1 (C(*C*H_3)$_3$), 60.6 (NCH), 70.0 (OCH_2), 84.0 (quat. C), 115.1 (Ar C), 126.7 (Ar C), 127.6 (Ar C), 128.0 (Ar C), 128.6 (Ar C), 131.2 (quat. Ar C), 136.8 (quat. Ar C), 149.3 (C=O), 153.0 (quat. Ar C), 158.0 (C=N), 169.6 (C=O).

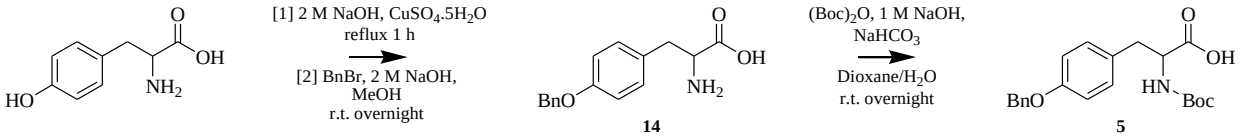
***tert*-Butyl 3-amino-2-(4-(benzyloxy)benzyl)-5-oxo-2*H*-pyrrole-1(5*H*)-carboxylate, 16**

To a mixture of *tert*-butyl 2-(4-(benzyloxy)-benzyl)-3-(hydroxyimino)-5-oxopyrrolidine-1-carboxylate **15** (3.64 g, 8.87 mmol) and MoO_3 (1.92 g, 13.31 mmol) in freshly distilled methanol (90 mL) was added cautiously $NaBH_4$ (1.68 g, 44.35 mmol). The resulting dark solution was stirred at room temperature for six hours, filtered and evaporated in vacuum. The residue was treated with 20% potassium hydroxide (KOH) and extracted with ethyl acetate (3 x 30 mL). The aqueous layer was saturated with brine and extracted with ethyl acetate (30 mL). The combined organic layers were washed with water (2 x 30 mL), brine (30 mL) and dried over anhydrous Na_2SO_4. Solvent removal in vacuum furnished crude yellow oil and subjected to column chromatography over silica gel (ethyl acetate/petroleum ether) to give an enamine 16 (0.66 g, 19%), IR ν cm^{-1}: 1748 (C=O), 1663 (C=O)**;** δ_H ($CDCl_3$, 300 MHz): 1.57 (9H, s, C(*C*H_3)), 3.05 (1H, dd, J = 9 Hz and J = 6 Hz, NCHCH*H*)), 3.29 (1H, dd, J = 3 Hz and J = 3 Hz, NCHC*H*H), 4.57 (1H, q, J = 3, 3 and 3 Hz, NCH), 4.63 (1H,s, C=CH), 4.96 (2H, s, OCH_2), 5.05 (2H, br s, NH_2), 6.86 (2H, d, J = 9 Hz, Ar H), 7.11 (2H, d, J = 9 Hz, Ar H), 7.30-7.41 (5H, m, Ar H); ^{13}C ($CDCl_3$, 75 MHz): 28.4 (C(CH_3)$_3$), 36.8 (NCH*C*H_2), 60.5 (NCH), 70.0 (quat. C), 82.1 (OCH_2), 90.8 (C=CH), 114.9 (Ar C), 127.2 (quat. Ar C), 127.6 (Ar C), 128.0 (Ar C), 128.6 (Ar C), 130.7 (Ar C), 136.9 (quat. C), 149.6 (C=O), 157.9 (quat. Ar C), 165.4 (C=O), 171.0 (C=O)

Results and Discussion

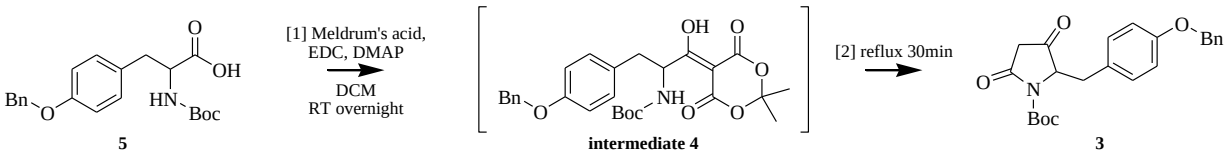
Based on the synthetic approach described in Scheme 1, we chose a readily available and cheap material, L-tyrosine as the starting material. We initially protected the hydroxyl and amino group in order to prevent any side reaction

from occurring [6]. The first step was the *O*-alkylation of *L*-tyrosine with benzyl bromide in 2 M sodium hydroxide (NaOH), copper sulfate solution and methanol to obtain the product **14** (71%) [7]. Treating the benzylated tyrosine with 1 M NaOH and sodium bicarbonate as a base in dioxane/water, followed by the addition of Boc anhydride ($(Boc)_2O$) successfully gave the desired Boc-protected amino acid **5** in 75% yield (Scheme 3) [7].



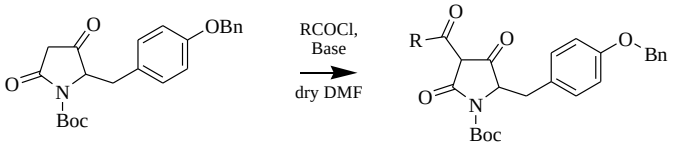
Scheme 3. *O*-Benzylation and *N*-Boc Protection of *L*-Tyrosine

Then we attempted to synthesize the tetramic acid template by employing Meldrum's acid activated by carboxyl activating agents, *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide (EDC), and 4-dimethylaminopyridine (DMAP) to produce an intermediate **4**. Subsequently, refluxing in ethyl acetate gave us the desired tetramic acid **3** in 70% yield (Scheme 4) [8].



Scheme 4. One-pot Condensation and Dieckmann Cyclisation of Protected *L*-Tyrosine

In further development towards the synthesis of the target compound **1**, we wanted to introduce an acyl group at C-3 position. In our first attempt to acylate tetramic acid **3** with acetyl chloride and potassium *tert*-butoxide in dimethylformamide (DMF) [9], no desired product was observed at the end of the reaction. The same thing happened when we used sodium hydride as a base. Instead, the starting material **3** was recovered but in low yield after purification using column chromatography. We then decided to use different acyl chlorides; fortunately, acylation of **3**, which was carried out in room temperature with potassium *tert*-butoxide and sodium hydride, separately, in anhydrous DMF, with benzoyl chloride and 2-butenoyl chloride, successfully gave C-3-acyltetramic acids, even though in low yields (Scheme 5 and Table 1).



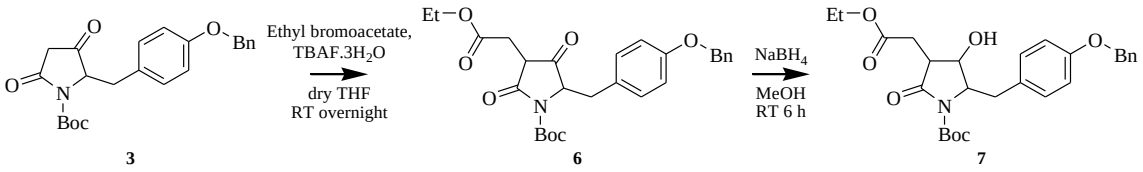
Scheme 5. Acylation of **3** with Different Acyl Chlorides and Bases

Table 1. Percentage Yields for Acylation of **3** with Different Acyl Chlorides and Bases

Product	Base	Reaction Condition	Yield (%)
4a	^{t}BuOK	0°C – r.t. 48 h	-
	NaH	-25°C – r.t. overnight	-
4b	^{t}BuOK	0°C – r.t. 48 h	37
	NaH	-25°C – r.t. overnight	25
4c	^{t}BuOK	0°C – r.t. 48 h	21
	NaH	-25°C – r.t. overnight	20

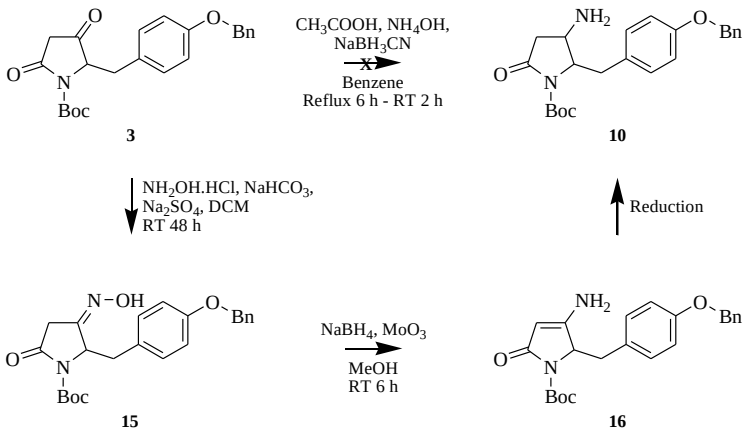
Consequently, the removal of Boc group could be performed by treating the reagent with strong acid such as hydrochloric acid (HCl) or trifluoroacetic acid (CF_3COOH), while the benzyl group could be removed *via* hydrogenation reaction.

For the synthesis of 3,4-fused γ-lactam-γ-lactone ring system **9**, we performed an insertion of the ethyl acetate functionality by treating the 4-hydroxybenzylated tetramic acid **3** with ethyl bromoacetate in anhydrous tetrahydrofuran (THF) with the presence of tetra-*n*-butylammonium fluoride trihydrate ($TBAF.3H_2O$) to produce the C-3 alkylated tetramic acid **6** in 10% yield [10]. Subsequently, the keto functionality was stereoselectively reduced with sodium borohydride ($NaBH_4$) in methanol to furnish its hydroxyl functionality **7** in 43% yield [11] (Scheme 6).



Scheme 6. Alkylation and Reduction of Tetramic Acid **3**

The formation of 3,4-fused γ-lactam-γ-lactone ring system **9** could then be completed by intramolecular cyclisation of **7** followed by removal benzyl and Boc groups, respectively. Meanwhile, the synthesis of 3,4-fused γ-lactam-γ-lactam ring system **13** was carried out by initially converting the keto to amine functionality of tetramic acid by reductive amination reaction. An attempt to react 4-hydroxybenzylated tetramic acid **3** in benzene with glacial acetic acid and ammonium hydroxide (NH_4OH) in a Dean-Stark apparatus [12] failed to produce the desired compound **10**, suggesting a stepwise reactions to transform the keto to amine at C-4 position. The tetramic acid **3** was then condensed with hydroxylamine hydrochloride ($NH_2OH.HCl$) in dichloromethane (DCM) to furnish an oxime **15** in 69%, followed by enamine formation upon treating with $NaBH_4$ and molybdenum trioxide (MoO_3) in 19% yield (Scheme 7) [12].



Scheme 7. Conversion of Keto to Amine Functionality

Similar to the lactam-lactone moiety **9**, the formation of 3,4-fused γ-lactam-γ-lactam ring system **13** could be completed by reduction of enamine **16** to produce the corresponding amine **10**, followed by alkylation at C-3 position, intramolecular cyclisation of **11** and removal of benzyl and Boc groups, respectively.

Conclusion

A short synthesis of 4-hydroxybenzylated 3-acyltetramic acids was accomplished by Meldrum's acid mediated reaction and tetramic acid cyclisation, followed by acylation reaction using different acyl chlorides and bases, while the key initial steps towards the synthesis of new 3,4-fused ring systems were identified and still under investigation. These synthetic strategies are potentially applicable to members of natural products with a similar ring moiety.

Acknowledgement

The authors acknowledge the generous support from Universiti Teknologi MARA as well as the financial support from Ministry of Higher Education Malaysia (FRGS grant 600-RMI/ST/FRGS 5/3/Fst (9/2010)).

References

1. Royles, B.J.L. (1995). Naturally Occurring Tetramic Acids: Structure, Isolation, and Synthesis. *Chem. Rev.*, 95: 1981 – 2001.
2. Casser, I., Steffan, B. & Steglich, W. (1987). The Chemistry of the Plasmodial Pigments of the Slime Mold *Fuligo septica* (Myxomycetes). *Angew. Chem. Int. Ed. Engl.*, 26: 586 – 587.
3. Steglich, W. (1989). Slime Moulds (*Myxomycetes*) as a Source of New Biologically Active Metabolites. *Pure & Appl. Chem*., 61: 281 – 288.
4. Ling, T., Potts, B.C. & Macherla, V.R. (2010). Concise Formal Synthesis of (-)-Salinosporamide A (Marizomib) Using a Regio- and Stereoselective Epoxidation and Reductive Oxirane Ring-Opening Strategy. *J. Org. Chem*., 75: 3882 – 3885.
5. Yamada, T., Sakaguchi, K., Shinada, T., Ohfune, Y. & Soloshonok, V.A. (2008). Efficient Asymmetric Synthesis of the Functionalized Pyroglutamate Core Unit Common to Oxazolomycin and Neooxazolomycin Using Michael Reaction of Nucleophilic Glycine Schiff Base with α,β-Disubstituted Acrylate. *Tetrahedron: Asymmetry*, 19: 2789 – 2795.
6. Hanson, J.R. (1999). Protecting Groups in Organic Synthesis. *Malden: Sheffield Academic Press*.
7. Caplar, V., Raza, Z., Katalenic, D. & Zinic, M. (2003). Syntheses of Amino Alcohols and Chiral C_2-Symmetric Bisoxazolines Derived from *O*-Alkylated *R*-4-Hydroxyphenylglycine and *S*-Tyrosine. *Croat. Chem. Acta*, 76(1): 23 – 36.
8. Hosseini, M., Kringelum, H., Murray, A. & Tonder, J.E. (2006). Dipeptide Analogues containing 4-Ethoxy-3-pyrrolid-2-ones. *Org. Lett*., 8(10): 2103 – 2106.

9. Toda, S., Nakagawa, S., Naito, T. & Kawaguchi, H. (1979). Bu-2313, A New Antibiotic Complex Active Against Anaerobes III. Semi-synthesis of Bu-2313 A and B, and their Analogs. *The Journal of Antibiotics*, 33: 173 – 181.
10. Page, P.C.B. Bulman Page, P. C., Hamzah, A. S., Leach, D. C., Allin, S. M., Andrews, D. M., & Rassias, G. A. (2003). Short and Versatile Route to a Key Intermediate for Lactacystin Synthesis. *Org. Lett*., 3: 353 – 355.
11. Fraga, C.A.M. & Barreiro, E.J. (1995). Studies toward the Diastereoselective Reduction of 2-Alkoxycarbonyl-2-allyl-cyclopentanone Derivatives with Boron Hydrides. *Synthetic Communications*, 25: 1133 – 1144.
12. Pinheiro, S. Ronaldo C. d S. J., Acácio S. d S., José W. de M. C., Estela M.F. M. and Antunes O.A.C. (2009). A General Approach for the Synthesis of 5-Substituted-4-Amino-Pyrrolidine-2-ones and 5-Substituted-4-Amino-3-Pyrrolin-2-ones. *Tetrahedron Letters*, 50: 2402 – 2404.