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SYNTHESIS OF 3-METHYL-5-NITROBENZYL β, β-DIKETOESTER AS A DERIVATIVE OF PACHYDERMIN, A TETRAMIC ACID FROM *Chamonixia pachydermis*

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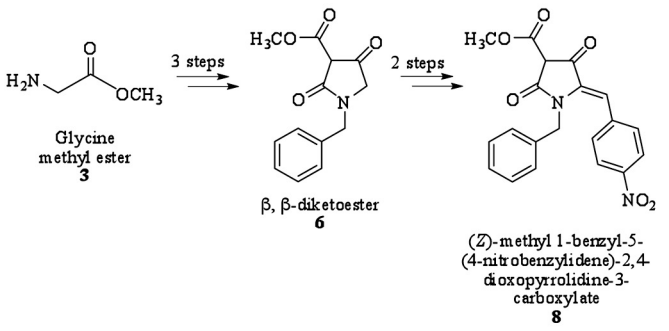
Abstract: Pachydermin, an oxylated tetramic acid with 3-chloro-4-hydroxyphenyl substituent, was isolated from *Chamonixia pachydermis* plant, a basidiomycete of New Zealand. Its degradation product exhibits antibacterial activity against *Bacillus subtilis*, and the derivatives of pachydermin are anticipated to have similar potentials. In this work, a novel approach to synthesize 3-methyl-5-nitrobenzyl β,β-diketoester was developed as a derivative of the natural product pachydermin. The synthetic route began with the synthesis of *N*-benzylated β,β-diketoester as the key structural moiety, from glycine methyl ester as the starting material. Subsequently, oxalyl subunit was inserted, as well as other acyl/alkyl subunits at C-3 position of the β,β-diketoester ring *via* acylation/alkylation reactions leading to the required intermediates towards pachydermin and its derivatives. Alkene functionalities at C-5 position could then be introduced using different alkyl or aryl aldehydes, with the aid of different bases which included diisopropylamine, NaH, Et_3N, K_2CO_3 as well as ionic liquids. Insertions of methyl and 4-nitrobenzylidene functionalities at C3- and C5-positions, respectively, were highlighted for the synthesis of the target derivative. Selective decarboxylation, ester hydrolysis and *N*-benzyl deprotection should lead to the required target compound and derivatives. All the synthesized compounds were confirmed by the mass spectroscopy (MS) and nuclear magnetic resonance (NMR) spectroscopy.

Keywords: *Chamonixia pachydermis*, pachydermin, tetramic acid.

Graphical Abstract



Introduction

The tetramic acids of pyrrolidinedione alkaloids have wide distribution in terrestrial and marine organism (Royles, 1995), and can be found in many biologically active natural products such as aurantosides, cylindramide, discodermide and reutericyclin (Figure 1) (Angawi *et al*., 2011). The natural products containing tetramic acid ring display remarkable diversity of bioactivities including anti-viral and anti-ulcerative properties, cytotoxicity and mycotoxicity, and fungicidal activity in tumor inhibition (Schobert

et al., 2008). Synthetic analogues of tetramic acids have been the subjects of clinical specifics in antibiotic areas. To date, the development of pyrrolidinedione alkaloids have been achieved by many synthetic efforts. Thus, invaluable and novel methodology to construct tetramic acids will contribute to the development of versatile chemical libraries with promising biological activities.

Pachydermin **1** which is an oxalylated tetramic acid (Figure 2) has been isolated from *Chamonixia pachydermis* of basidiomycete (Lang *et al*., 2006). The filamentous fungus is found in *Nothofagus* forest in Australia and New Zealand. The structure of **1** is analysed from its degradation product, 5-(3-chloro-4-hydroxy- benzylidene) tetramic acid **2** because compound **1** could not be directly accessed by NMR method (Figure 2). The purification of **1** is also difficult to achieve even in weak acidic condition of trifluoroacetic acid (0.05% TFA) which is used in the preparative HPLC. Thus, a complete conversion into **2** is performed by heating **1** in 2 M HCl and eventually the degradation of product **2** is identified and the structure for natural product **1** is proposed (Lang *et al*., 2006).

The structure of the 3-chloro-4-hydroxyphenyl substituent at the C5-position of **1** has been confirmed *via* NMR and MS analyses. However, the oxalyl tetramic acid part with the molecular formula of $C_6H_3NO_2$ could not be accounted for. For this structural subunit, no associated sharp proton signals are observed; and only four low-field carbons and two sp^2-carbons are present in the ^{13}C NMR spectrum. In order to gather more information on the structure, the structure of **1** is deduced from **2** by referring to the loss of C_2O_3 from the parent structure *via* a concerted decarbonylation-decarboxylation reaction of its keto tautomer as depicted in Scheme 1 (Figure 3).

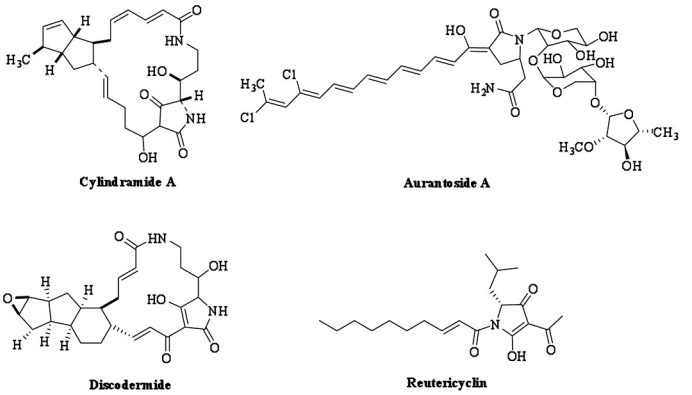


Figure 1: Examples of natural products containing tetramic acid

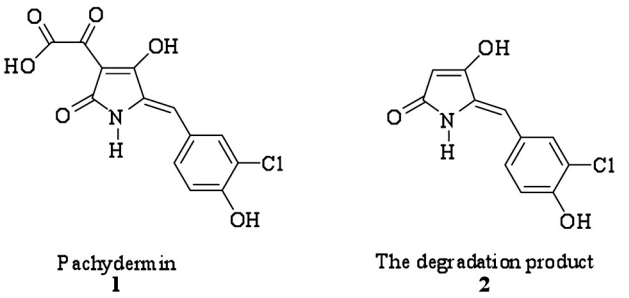


Figure 2: Structures of pachydermin **1** and its degradation product **2**

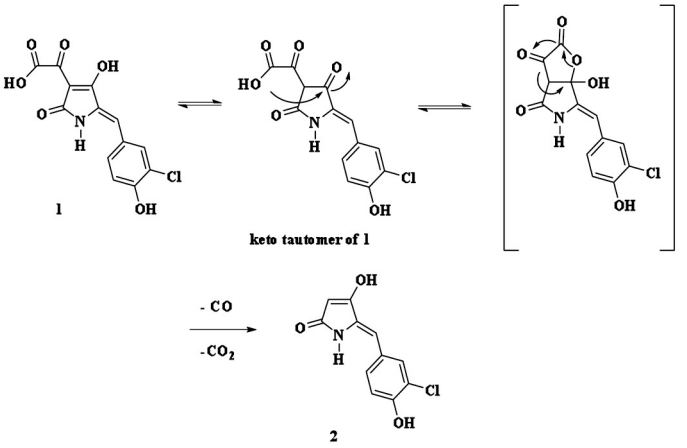


Figure 3: Decarbonylation-decarboxylation of **1** to **2 (**Scheme 1**)**

Although the 3-chloro-4-hydroxyphenyl substituent is a normal structural feature in many bacterial and fungal natural products (Davis *et al*., 2005), the oxalylated tetramic acid moiety is l a distinctive structural constituent of **1**. It is also known that many natural compounds with methyl-substituted tetramic acid ring display equally important pharmaceutical and therapeutic ability. Such compounds include a protein phosphatase inhibitor, Dysidamide C and antiproteasome drugs, lactacystin and salinosporamide (Ling *et al*., 2007). Therefore, the objective of this study was to synthesize a novel derivative of **1** with its highly anticipated similar bioactivity.

Materials and Methods

General procedures: ^{1}H-NMR and ^{13}C-NMR spectra were recorded in deuterated solvents with tetramethylsilane as an internal standard measured on JEOL NMR instrument at 400 MHz and 100 MHz, respectively. Chemical shifts were expressed in δ (parts per million (ppm) units.

Methyl 2-(benzylamino) acetate (4). To a stirred solution of glycine methyl ester (5.00 g, 39.82 mmol, Sigma-Aldrich Co., USA) in acetonitrile (200 mL), potassium carbonate (13.76 g, 99.55 mmol) was added. The solution was cooled at 0 °C, and benzyl bromide (4.72 mL, 39.82 mmol, Sigma-Aldrich Co., USA) was added. After stirring at room temperature for 12 h, the reaction mixture was quenched with water (100 mL). The reaction mixture was extracted with ethyl acetate (EA) (3 × 40 mL) and dried over anhydrous Na_2SO_4, to give the crude residue which was later purified by column chromatography on silica gel (petroleum ether/ diethyl ether, 50/50) as colourless oil in 3.92 g (55%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.97 (s, 1H, NH), 3.42 (q, 1H, J = 6.0 Hz, CH), 3.68 (d, 1H, J = 15.0 Hz, CH_2Ph), 3.74 (s, 3H, OCH_3), 3.82 (d, 1H,J = 12.0 Hz, CH_2Ph), 7.23–7.35 (m, 5H, aromatic H); ^{13}C NMR ($CDCl_3$, 100 MHz): 51.81 (CH_2Ph), 51.98 (OCH_3), 55.89 (CH), 127.11– 128.44 (aromatic C), 139.68 (quat. aromatic C), 176.21 (C=O).

Methyl 3-(benzyl (2-methoxy-2-oxoethyl) amino)-3-oxo-propanoate (5). To a solution of methyl 2-(benzylamino) acetate, **4** (5.00 g, 27.89 mmol), methyl malonyl chloride (2.82 mL, 26.30 mmol, Sigma-Aldrich Co., USA) in anhydrous benzene (50 mL) was added and the mixture was refluxed under nitrogen for 3 hours. The solvent was removed and the residual product was triturated with ether, washed with saturated $NaHCO_3$ and brine, dried over anhydrous Na_2SO_4 and evaporated again to give a colorless oil in 5.14 g (70%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 3.44 (q, 2H, J = 15.0 Hz, CH_2), 3.71 (d, 6H, J = 12.0 Hz, 2 × OCH_3), 4.51–4.76 (m, 3H, CH_2Ph and CH), 7.21–7.41 (m, 5H, aromatic H); ^{13}C NMR ($CDCl_3$, 100 MHz):

14.72 (CH_3), 41.32 (CH_2), 50.65 (CH_2Ph), 52.49 (OCH_3), 54.30 (OCH_3), 126.37–128.96 (aromatic C), 136.41 (quat. aromatic C), 167.18 (C=O), 167.70 (C=O), 171.79 (C=O).

Methyl 1-benzyl-2,4-dioxopyrrolidine-3-carboxylate (6). Potassium *tert*-butoxide (2.00 g, 17.91 mmol, Sigma-Aldrich Co., USA) in toluene (40 mL) was added into methyl-3-(benzyl (2-methoxy-2-oxoethyl) amino)-3 oxopropanoate, **5** (5.00 g, 17.91 mmol), that has been dissolved in toluene (35 mL), and stirring was continued for one hour at room temperature. The resulting solution was diluted with water (20 mL) and extracted. The combined aqueous extracts were acidified with concentrated HCl to form white precipitate and filtered as white powder 3.81 g (86%). ^{1}H NMR data indicated that **6** exists as its enol tautomer. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 3.94 (s, 4H, OCH_3 and CH), 4.08 (d, 1H, J = 18.0 Hz, CH_2Ph), 5.14 (d, 1H, J = 15.0 Hz, CH_2Ph), 7.23–7.33 (m, 5H, aromatic H); ^{13}C nmr ($CDCl_3$, 100 MHz): 40.52 (CH_2Ph), 50.86 (CH-CH_3), 52.23 (OCH_3), 61.72 (CH), 127.71–128.98 (aromatic C), 135.22 (quat. aromatic C), 168.05 (C=O), 168.48 (C=O), 206.72 (C=O).

Methyl-1-benzyl-3-methyl-2,4-dioxo-pyrrolidine-3-carboxylate (7). To a stirred solution of methyl 1-benzyl-2, 4-dioxopyr-rolidine-3-carboxylate, **6** (2.00 g, 8.09 mmol) in tetrahydrofuran (THF) (40 mL), tetrabutylammonium fluoride (TBAF) 1 M in THF (9.71 mL, 9.71 mmol, Sigma-Aldrich Co., USA) was added. The reaction mixture was stirred until all solids were dissolved. Methyl iodide (1.52 mL, 24.27 mmol, Merck Chemical Co., German) was then added and the reaction mixture was stirred overnight. The solvent was removed and the crude product was then chromatographed on silica gel (petroleum ether/ ethyl acetate, 60/40) to produce a colourless oil in 1.86 g (70%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.51 (s, 3H, CH_3), 3.70 (s, 3H, J = 12.0 Hz, OCH_3), 4.00–4.13 (m, 3H, OCH2 and CH), 4.30 (d, 1H, J = 15.0 Hz, CH_2Ph), 5.18 (d, 1H, J = 15.0 Hz, CH_2Ph), 7.28–7.38 (m, 5H, aromatic H); ^{13}C NMR ($CDCl_3$, 100 MHz): 13.99 (CH_3), 15.30 (CH_3), 36.53 (CH_2), 44.64 (CH_2Ph), 53.65 (OCH_3), 59.49 (CH), 60.46 (OCH_2), 61.77 (quat. C), 127.90–128.77 (aromatic C), 135.04 (quat. aromatic C), 167.74 (C=O), 170.32 (C=O), 204.96 (C=O).

(Z)-methyl1-benzyl-3-methyl-5-(4-nitrobenzylidene)-2,4-dioxopyrrolidine-3-carboxylate (8). A mixture of methyl 1-benzyl-3-methyl-2,4-dioxopyrrolidine-3-carboxylate, **7** (0.30 g, 1.15 mmol) and diisopropylamine (0.64 mL, 4.60 mmol, Sigma-Aldrich Co., USA) in dry THF (15 mL) was stirred at 60 °C under nitrogen for 45 minutes. 4-Nitrobenzaldehyde (0.35 g, 2.30 mmol, Sigma-Aldrich Co., USA) in 5 mL of THF was added to the resulting solution and refluxed. After 5 h, 10 mL of H_2O was added and the mixture was refluxed overnight. The reaction mixture was evaporated under reduced pressure, the crude product was partitioned against ether (3x50 mL) and the combined organic phases were washed with brine (20 mL), and then dried over anhydrous $MgSO_4$, filtered and evaporated to produce crude extract. The crude extract was purified by column chromatography on silica gel (petroleum ether/ethyl acetate, 60/40) to give a yellowish oil in 0.04 g (15%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.51 (s, 3H, CH_3), 3.70 (s, 3H, J = 12.0 Hz, OCH_3), 6.60 (s, 1H,CH), 4.30 (d, 1H, J = 15.0 Hz, CH_2Ph), 5.18 (d, 1H, J = 15.0 Hz, CH_2Ph), 7.28–7.38 (m, 5H, aromatic H), 8.03 (d, 2H, aromatic H), 8.21 (d, 2H, aromatic H); ^{13}C NMR ($CDCl_3$, 100 MHz): 13.99 (CH_3), 15.30 (CH_3), 36.53 (CH_2), 44.64 (CH_2Ph), 53.65 (OCH_3), 59.49 (CH), 60.46 (OCH_2), 61.77 (quat. C), 127.90–128.77 (aromatic C), 135.04 (quat. aromatic C), 123.8 – 129.0 (aromatic C), 140.3 (quat. aromatic C), 147.0 (quat. aromatic CNO_2), 167.74 (C=O), 170.32 (C=O), 204.96 (C=O).

Results and Discussion

The synthetic route began with benzylation of glycine methyl ester, **3** with benzyl bromide in the presence of K_2CO_3 in acetonitrile to give *N*-benzylated ester derivative of glycine, methyl 2-(benzylamino) acetate, **4** in 55% yield (Scheme 2). Accordingly, **4** was coupled with methyl malonyl chloride in dry benzene

via condensation reaction to give a diester intermediate, **5** in 70% yield. Dieckmann cyclisation of **5** was then performed with potassium *tert*-butoxide in toluene as the base at room temperature which produced the required tetramic acid moiety, *N*-benzylated β,β-diketoester, **6** in 86% yield.

In order to generate an intermediate with methyl substituent at C3-position of the tetramic acid, **6** was alkylated with methyl iodide in the presence of TBAF as a phase transfer catalyst as well as a base, in THF solvent according to Page's protocol (Page, 2003) to give the expected precursor **7** in 70% yield. The structure of **7** was confirmed by NMR analyses. Alternatively, different bases such as NaH, Et_3N, K_2CO_3 as well as ionic liquids, with different alkyl and acyl halides were employed to further explore

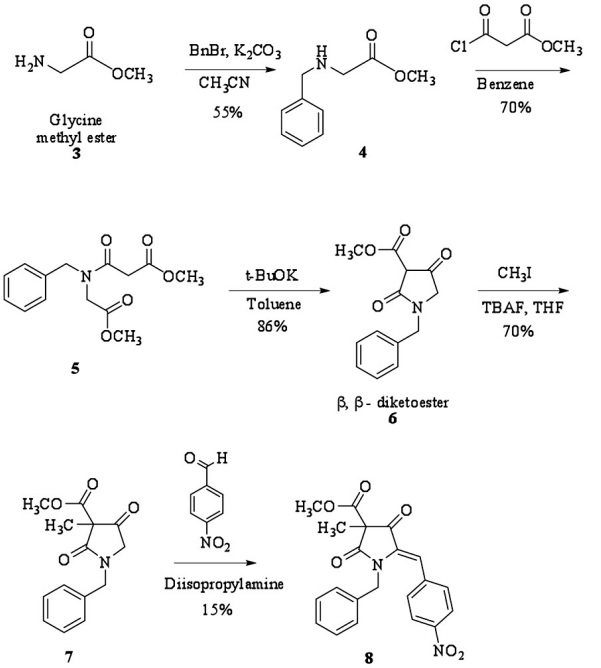


Figure 4: Synthetic route to compound **8** (Scheme 2)

Table 1: The Alkylation reaction at C3 position of N-benzylated β, β-diketoester

Electrophile	Reaction Condition	Yield %
C_6H_5COCl (1.2 eq)	TBAF Trihydrate (1.5 eq), Dry THF, stir 24 hours	3 – *C*-acylated
C_6H_5COCl (1.5 eq)	TBAF in 1M THF (2.0 eq), Dry THF, stir 24 hours	20 – *C*-acylated 14 – O-acylated
CH_3COCl (1.5 eq)	TBAF in 1M THF (2.0 eq), Dry THF, stir 24 hours	15 – *C*-acylated
$CH_3CH_2OCOCOCl$ (1.2 eq)	TBAF trihydrate (1.5 eq), Dry THF, stir 24 hours	Recover starting material
	TBAF in 1M THF (1.5 eq), Dry THF, stir 24 hours	7 – *C*-acylated
	NaH (1.5 eq), Dry benzene, relux 5 hours	Recover starting material
$CH_3CH_2OCOCOCl$ (3.0 eq)	TBAF in 1M THF (2.0 eq), Dry THF, stir 24 hours	10 – *C*-acylated
	NaH (2.0 eq), Dry DMF, r.t, Stir 5 hours	Recover starting material
$CH_3CH_2OCOCOCl$ (6.0 eq)	TBAF in 1M THF (2.0 eq), Dry THF, stir 4 days	35 – *C*-acylated

Table 2: The Alkylation reaction at C3 position of N-benzylated β, β-diketoester

Electrophile	Reaction Condition	Yield %
CH_3I (3.0 eq)	TBAF trihydrate (1.5 eq), THF, stir at r.t, 24 hours	70
	K_2CO_3 (1.2 eq), CH_3CN, reflux 5 hours	15
CH_3CH_2I (3.0 eq)	TBAF trihydrate (1.5 eq), THF, stir at r.t, 24 hours	40
$CH_3CH_2CH_2I$ (3.0 eq)	TBAF trihydrate (1.5 eq), THF, stir at r.t, 24 hours	20
$CH_3CH_2OCOCH_2I$ (3.0 eq)	TBAF trihydrate (1.5 eq), THF, stir at r.t, 24 hours	35
CH_2=$CHCH_2Br$ (3.0 eq)	TBAF trihydrate (1.5 eq), THF, stir at r.t, 24 hours	30
CH_3OOCH_2Br (3.0 eq)	TBAF trihydrate (1.5 eq), THF, stir at r.t, 24 hours	40
C_6H_5CH=$CHCH_2Br$ (3.0 eq)	TBAF trihydrate (1.5 eq), THF, stir at r.t, 24 hours	15
$CH_3CH_2OCH_2Cl$ (3.0 eq)	TBAF trihydrate (1.5 eq), THF, stir at r.t, 24 hours	5

the feasibility of the C3-acylation (Table 1) and C3-alkylation reactions (Table 2).

Having successfully inserted methyl group at C3-position, the next attempt was to introduce the alkene functionality at the C5-position. Employing the previously reported method (Bathich *et al.,* 2011), the strategy was achieved by reacting with di*iso*propylamine and 4-nitrobenzaldehyde in THF to yield 15% of the essential precursor **8**.

In future development towards the synthesis of a derivative of pachydermin, the methyl ester functionality at the C3-position has to be removed. Selective removal of the methyl ester or demethoxycarbonylation can be accomplished using LiI in DMF or any other salt-solvent systems such as KCN in HMPA, LiI in pyridine and NaCl in DMSO. Finally, removal of the *N*-benzyl protecting group using hydrogen gas will yield the required methyl derivative of the natural pachydermin.

Conclusion

A derivative of pachydermin [(Z)-methyl 1-benzyl-3-methyl-5-(4-nitrobenzylidene)-2,4-dioxopyrrolidine-3-carboxylate from glycine methyl ester was successfully obtained *via* a five-sequential synthetic step with an overall yield of 3.54%.

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