# Synthesis of 20-*N*-Methylpurpuramine E, an Antitubercural Metabolite from *Pseudoceratina* sponge

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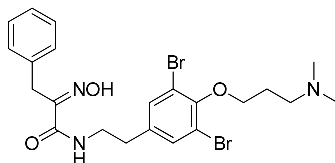
**Abstract**- 20-*N*-Methylpurpuramine E has been successfully synthesized in 2 steps in good yield from readily available starting materials. Structural elucidation has been confirmed through direct comparison with spectroscopic data of isolated natural product. 20-*N*-Methylpurpuramine E has been shown to have MIC = 5 μg $mL^{-1}$ against *Mycobacterium bovis*.

**Keywords**: marine sponge metabolites, bromotyramine, bromotyrosine, antituberclar.

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## Introduction

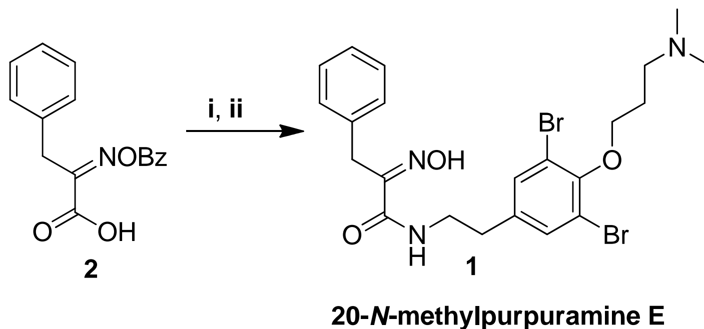
Bromotyrosine-derived marine natural products from the order *Verongida* show unique and diverse biological activities that include antimicrobial, antiviral, anti-angiogenic, antifouling and enyzme modulatory properties.[1] These structurally diverse secondary metabolites accumulated in concentrations of up to 10% sponge dry weight, can act in rapid activated chemical defense mechanisms against a large number of organisms in aqueous environments.[2] Due to substantial and immediate dilution effects once outside the host organism such compounds display exceptional potency toward predators coupled with low toxicity towards the host. This makes these compounds attractive targets for systematic biological evaluation and total synthesis.

20-*N*-Methylpurpuramine E (**1**) is an analogue of natural occurring marine sponge Purpuramine E and has been isolated (13.1 mg from 480 g wet weight) from the Okinawan sponge *Pseudoceratina purpurea*.[3] The natural product was identified through the use of $^{1}$H and $^{13}$C NMR spectroscopy, as well as HRMS data and, due to the scarcity of 20-*N*-Methylpurpuramine E (**1**), was only tested against HeLa $S_3$ cells in which it exhibited weak cytoxicity $IC_{50} = 4.3$ µg $mL^{-1}$.

Following on from our previous synthetic endeavors,[4] we have investigated the synthesis and biological evaluation of 20-*N*-Methylpurpuramine E (**1**) for antitubercular activity against *M. bovis*.

## Results and Discussion

The starting material for the synthesis of **1** was the known 2-[(*E*)-benzyloximino]-3-phenylpropionic acid (**2**).[5] Simple carbodiimide coupling with Purpurealidin E,[6] followed by hydrogenation of the benzyl protecting group, gave **1** in 57% yield over the two steps.

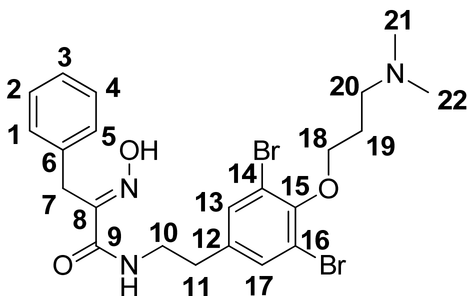



**Reagents and Conditions**:

(i) Purpurealidin E, EDC, HOBt, $Et_3N$, $CH_2Cl_2$, room temp **67%**; (ii) Pd-C/$H_2$, Dioxane/AcOH **85%.**

Structure elucidation was achieved by direct comparison of the natural versus the synthetic product (**Table 1**) and excellent spectral correlation was observed, with the $^{13}C$ chemical shift of C-7 at 30.1 ppm suggesting an *E*-geometry for the oxime.[7]

**Table 1. NMR and MS Data for 1**




| Position | $^{1}$H NMR | | $^{13}$C NMR | |
|---|---|---|---|---|
| | A | B | A | B |
| 1, 5 | 7.21 (m, 2H) | 7.23 (m, 2H) | 129.9 | 130.0 |
| 2, 4 | 7.20 (m, 2H) | 7.19 (m, 2H) | 129.3 | 129.4 |
| 3 | 7.13 (m, 1H) | 7.16 (m, 1H) | 127.2 | 127.3 |
| 6 | | | 138.1 | 138.1 |
| 7 | 3.88 (s, 2H) | 3.91 (s, 2H) | 29.9 | 30.1 |
| 8 | | | 153.2 | 153.2 |
| 9 | | | 166.0 | 166.0 |
| 10 | 3.41 (t, $J$ = 6.8 Hz, 2H) | 3.31 (t, $J$ = 6.8 Hz, 2H) | 41.3 | 41.4 |
| 11 | 2.72 (t, $J$ = 6.8 Hz, 2H) | 2.75 (t, $J$ = 6.8 Hz, 2H) | 35.2 | 35.2 |
| 12 | | | 140.3 | 140.2 |
| 13, 17 | 7.43 (s, 2H) | 7.40 (s, 2H) | 134.4 | 134.4 |
| 14, 16 | | | 118.7 | 118.8 |
| 15 | | | 152.1 | 152.3 |
| 18 | 3.45 (t, $J$ = 7.2 Hz, 2H) | 3.45 (t, $J$ = 6.9 Hz, 2H) | 71.0 | 71.4 |
| 19 | 2.26 (m, 2H), | 2.26 (m, 2H), | 26.3 | 26.9 |
| 20 | 4.07 (t, $J$ = 5.6 Hz, 2H) | 4.06 (t, $J$ = 5.7 Hz, 2H) | 57.0 | 57.0 |
| 21, 22 | 2.94 (s, 6H) | 2.80 (s, 6H) | 43.6 | 44.0 |
| MS | HR-EI $[MH]^+$ ($C_{22}H_{27}{}^{79}Br_2N_3O_3$)<br>Calculated 540.0497<br>Observed 540.0485 | HR-EI $[MH]^+$ ($C_{22}H_{27}{}^{79}Br_2N_3O_3$)<br>Calculated 540.0497<br>Observed 540.0491 | | |

**A** – Suenaga, K. *et al.*; *Bull. Chem. Soc. Jpn.* **2008**, 81(8), 1026-7. ($^{1}$H 400 MHz, $^{13}$C 100 MHz, $CD_3OD$)
**B** – The results we obtained. ($^{1}$H 500 MHz, $^{13}$C 125 MHz, $CD_3OD$)

*Antimycobacterial Activity*

MICs against mycobacteria were determined using a spot culture growth inhibition assay,[8] which has been previously shown to have identified antimycobacterial compounds from both natural products[9] and novel synthetic compounds.[10] **Figure 1** illustrates a representative MIC determination using this assay for compound **5,** demonstrating MIC of 5 µg $mL^{-1}$ for *M. bovis* BCG.

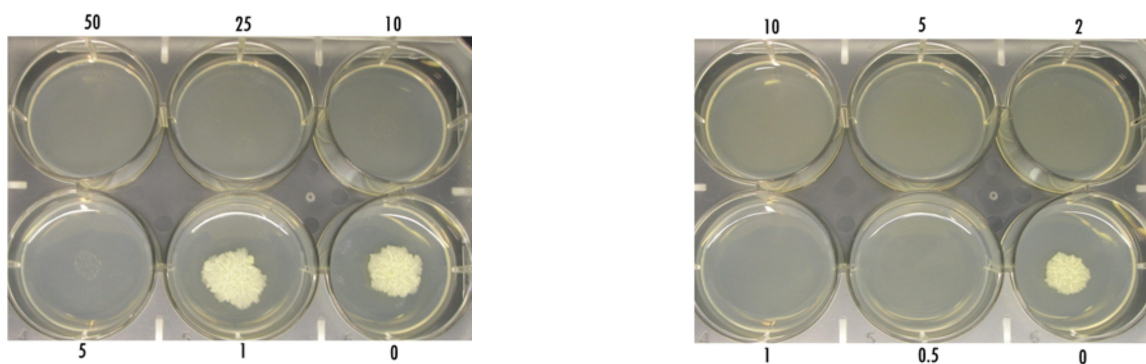



**Figure 1.** The plates show a representative assay of the growth of *M. bovis* BCG on 7H10 agar at 37 °C in the presence of different concentrations of compound **1** and Isoniazid. The MIC values for compound **1** (left) and Isoniazid (right) are 5 μg $mL^{-1}$ and 0.5 μg $mL^{-1}$, respectively, for *M. bovis* BCG. Mycobacterial species were grown in Middlebrook 7H9 broth medium supplement with ADC at 37 °C. 103 cells were spotted on the centre of each well of a six well plate containing Middlebrook 7H10 agar medium, supplemented with 10% OADC, and different concentrations of **1** and Isoniazid (0, 0.1, 1, 10, 25, and 40 μg $mL^{-1}$). Images of cultures that grew as spots were taken on the $14^{th}$ day after inoculation using a digital camera.

There is good correlation ($r^2$=1) between the MIC for *M. bovis* BCG and the MIC for *M. tuberculosis* H37Rv, an observation that has been reported previously using the spot growth inhibition assay.[10b] Further testing on *M. tuberculosis* H37Rv is ongoing and results of these further investigations will be reported in due course. In summary, this paper describes the first formal synthesis of the marine sponge secondary metabolite 20-*N*-Methylpurpuramine E (**1**) from readily available starting materials and provides the first evidence of its biological activity against *M. bovis* BCG.

**Experimental**

***O*-Benzyl-20-*N*-methylpurpuramine-E (2)**

3-Phenyl-2-benzyloxyiminopropanoic acid[5] (0.80 g, 2.97 mmol), Purpurealidin E[6] (1.10 g, 2.97 mmol), EDC.HCl (0.85 g, 4.46 mmol), HOBt (0.40 g, 2.97 mmol) and $Et_3N$ (1.3 mL, 8.90 mmol) in dry $CH_2Cl_2$ (10 mL) were stirred at room temperature until no starting material remained by TLC ($SiO_2$, $CH_2Cl_2$/MeOH, 5:2).The reaction mixture was diluted with $H_2O$ (25 mL), extracted with EtOAc (3 x 25 mL), washed with sat. NaCl (25 mL), dried over anhydrous $Na_2SO_4$, and the solvent removed *in vacuo* to give orange gum which was purified by column chromatography ($SiO_2$, $CH_2Cl_2$/MeOH, 5:2) to give a colourless gum (1.24 g, 67 %); IR (Diamond) $\upsilon_{max}$ 1642 (C=O) $cm^{-1}$. $^1H$ NMR (300 MHz, $CD_3OD$) δ 7.43 (s, 2H), 7.38-7.31 (m, 5H), 7.29-7.15 (m, 5H), 5.26 (s, 2H), 4.05 (t, $J$ = 6.3 Hz, 2H), 3.92 (s, 2H), 3.47 (t, $J$ = 6.9 Hz, 2H), 2.78 (t, $J$ = 6.9 Hz, 2H), 2.69 (t, $J$ = 7.8 Hz, 2H), 2.32 (s, 6H), 2.11(m, 2H); $^{13}C$ NMR (75 MHz, $CD_3OD$) δ 162.2 (qC), 151.1 (qC), 150.1 (qC), 136.8 (qC), 135.6 (qC), 134.7 (qC), 131.5 (CH), 127.2 (CH), 126.6 (CH), 126.6 (CH), 126.3 (CH), 126.3 (CH), 124.6 (CH), 116.1 (qC), 75.5 ($CH_2$), 69.8 ($CH_2$), 54.7 ($CH_2$), 42.6, $CH_3$), 38.6 ($CH_2$), 32.3 ($CH_2$), 28.1 ($CH_2$), 26.1 ($CH_2$); LR-EI (+ve) Calcd.for $C_{29}H_{34}{}^{79}Br^{81}BrN_3O_3$ $[MH]^+$: 632.1, found 632.2.

**20-*N*-Methylpurpuramine E (1)**

A solution of *O*-Benzyl-20-*N*-methylpurpuramine-E (0.40 g, 0.64 mmol) in AcOH (1.5 mL) and dioxane (1.5 mL) was hydrogenated over Pd/C (10 wt%, 0.10 g) at room temperature until no starting material remained by TLC ($SiO_2$, EtOAc/MeOH, 5:2).After filtration, the solution was basified with sat. $NaHCO_3$, extracted with EtOAc (3 x 25 mL), washed with sat. NaCl solution (25 mL), dried over an. $Na_2SO_4$ and solvent removed *in*

*vacuo* to give orange gum which was purified by column chromatography ($SiO_2$, EtOAc /MeOH, 5:2) to give yellow gum (0.31 g, 91 %). IR (Diamond) $\upsilon_{max}$ 3389 (OH), 1672 C=O), $cm^{-1}$. $^1H$, $^{13}C$ NMR and HR- MS see **Table 1**

**Determination of MIC *M. bovis* BCG**

*M. bovis* BCG was grown in 100 mL roller bottles at 37 °C incubator, with rotating at 2 rpm in Middlebrook 7H9 medium supplemented with 10% (v/v) albumin-dextrose-catalase (ADC; BD) and 0.05% Tween 80 until the mid-exponential phase ($1OD_{600}$). For the quality control of the mycobacterial culture, *M. bovis* BCG was stained with a modified Ziehl-Neelsen staining protocol using a Tb-color kit, Bund Deutscher Hebammen Laboratory (Karlsruhe, Germany) according to manufacturer's procedure.

For the Spot-Culture Inhibition Assay, mycobacterial cultures were serially diluted up to 105 CFU $mL^{-1}$. Then, 10 µL of that culture was spotted in 5 mL of Middlebrook 7H10 agar medium, supplemented with 10% (v/v) OADC in a six-well plate containing various concentrations of compounds, and incubated at 37 °C for 2 weeks. A well containing DMSO (5 µL) instead of compound was used as a positive control. The minimum inhibitory concentration (MIC) was determined as the lowest concentration at which there is no growth of mycobacteria in the well.

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