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An improved synthesis of psammaplin A

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Abstract—The marine natural product, psammaplin A, was first isolated from the *Psammaplinaplysilla* sponge in 1987. Since that time, psammaplin A has shown a wide spectrum of biological activities that include enzyme inhibitory activities resulting in antibacterial and antitumor effects. An improved synthesis of psammaplin A has been developed, making the compound more easily accessible for further biological evaluations. In this context, we find that psammaplin A is an effective DNA methyltransferase inhibitor in vitro but fails to alter genomic DNA methylation levels in treated human cancer cells.

Psammaplin A (PsA) is a bromotyrosine-derived, symmetrical conjugate of cystamine, which was first isolated from the *Psammaplinaplysilla* sponge in 1987.[1] In the ensuing years, psammaplin A has been found to induce a wide spectrum of biological effects.[2–13] Psammaplin A has been shown to impede angiogenesis as well as bacterial and tumor cell growth.[4,6–8,10,11] Notably, psammaplin A inhibits the activities of several key enzymes in a number of distinct prokaryotic and eukaryotic systems including those involved in epigenetic control of gene expression, DNA replication, angiogenesis, and microbial detoxification.[2,4–6,10–13] Table 1 contains a list of enzymes which are currently known to be susceptible to inhibition by psammaplin A.

The novelty of psammaplin A's structure and the diversity of its known biological targets have prompted its consideration as a lead compound for drug design. An extensive library of heterodimeric disulfide analogs of psammaplin A was prepared by combinatorial exchange synthesis and then screened against methacillin-resistant *Staphylococcous aureus*, leading to the identification of several novel lead structures with increased antibiotic potency.[7,8]

Psammaplin A has been obtained from its natural source by tedious extraction and separation procedures.[1,3,9,14] In addition, two synthetic preparations of psammaplin A have been reported.[7,15] We have devised an improved synthetic route which should make psammaplin A more accessible for biological studies.

The first reported synthesis of psammaplin A by Hoshino et al. uses 3-bromotyrosine **1** as the starting material (Scheme 1).[15] Bromotyrosine **1** is converted to the azalactone **2** via reflux in trifluoroacetic anhydride. Treatment of **2** with aqueous TFA provides 3-(3-bromo-4-hydroxyphenyl)-α-oxo-propanoic acid **3.** The oxime derivative **4** is formed by treating **3** with hydroxylamine. Analogs of the oximino acid **4** have been prepared recently using similar chemistry.[16–18] Psammaplin A is obtained by coupling **4** with cystamine in dioxane through activation of the carboxylate with DCC and triethylamine. The overall yield of psammaplin A is 22.5%, based on 3-bromotyrosine as starting material.

A recently reported six-step synthesis of psammaplin A (Scheme 2) uses tyrosine **5** as starting material to obtain the α-keto acid **3** in a similar reaction sequence as Hoshino et al.[7] This intermediate is converted to its *O*-(tetrahydro-2*H*-pyran-2-yl)-hydroxylamine (THP) derivative **6** which is coupled with cystamine to give **7**, the bis-(*O*-THP) derivative of psammaplin A. Acid catalyzed deprotection of **7** gives the desired product, psammaplin A, in an overall yield of 36%.

Keywords: Psammaplin A; 5-Bromo-psammaplin A; DNA methylation; DNA methyltransferase; DNA methyltransferase inhibitor; Cancer cells.
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Table 1. Enzyme targets of psammaplin A inhibition

Enzyme	Reference
Aminopeptidase N	Shim et al.[11]
Chitinase	Tabudravu et al.[13]
Farnesyl protein transferase	Shin et al.[12]
DNA gyrase	Kim et al.[5]
DNA polymerase α-primase	Jiang et al.[2]
DNA methyltransferase	Pina et al.[10]
Histone deacetylase	Pina et al.[10]
Leucine aminopeptidase	Shin et al.[12]
Mycothiol-S-conjugate amidase	Nicholas et al.[6]
Topoisomerase II	Kim et al.[4]

Our simplified synthesis of psammaplin A utilizes 4-hydroxyphenylpyruvic acid **8**, a commercially available starting material. Conversion of **8** to psammaplin A entails three synthetic reactions (i.e., bromination, oximation, and coupling) that can potentially be performed in different sequences. We investigated three possible reaction routes:

I. 4-Hydroxyphenylpyruvic acid → **bromination** → **oximation** → **coupling** → *psammaplin A*

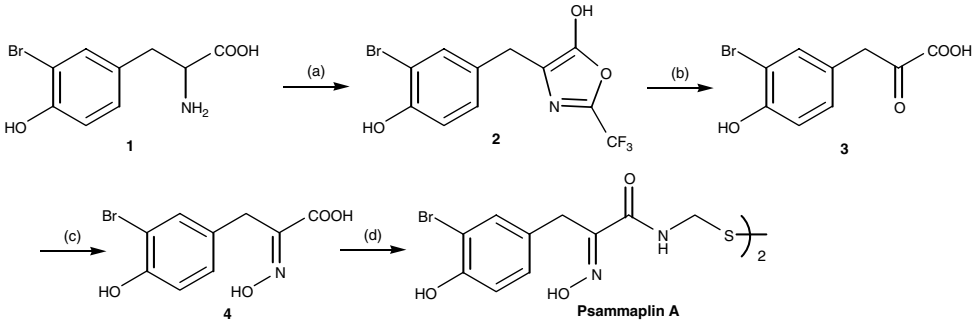
II. 4-Hydroxyphenylpyruvic acid → **oximation** → **coupling** → **bromination** → *psammaplin A*

III. 4-Hydroxyphenylpyruvic acid → **oximation** → **bromination** → **coupling** → *psammaplin A*

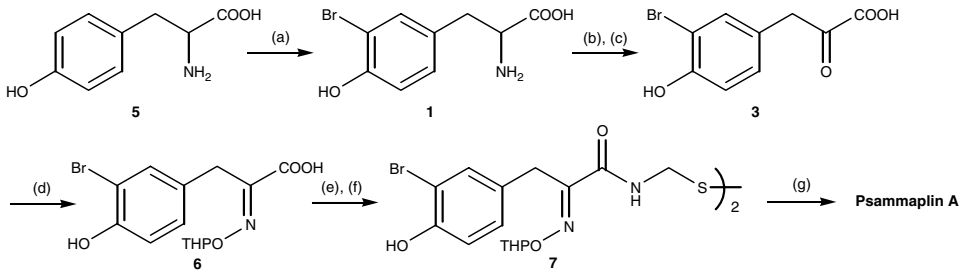
Attempts to brominate **8** were unsuccessful due to non-chemoselective bromination and route **I** was abandoned at the outset. Compound **9**,[19] the oxime derivative of **8**, was routinely prepared and coupled with cystamine to give the bis-(3-debromo)-derivative of psammaplin A. Attempted bromination of this coupled intermediate resulted in a complex mixture of brominated products, eliminating route **II** from further consideration. However, the oxime **9** was successfully brominated to produce **4**,[15,17] which was then coupled with cystamine to give psammaplin A in 43% overall yield (Scheme 3).

Bromination of **9** produced a small amount of the 3,5-dibrominated oxime acid **10**[16] (16% yield). Compound **10** was separated from **4** (81% yield) by Sephadex LH20 chromatography and then coupled with cystamine to give compound **11**, the 3,5-dibromo-analog of psammaplin A (5-Br-psammaplin A, 5-BrPsA).[7,9]

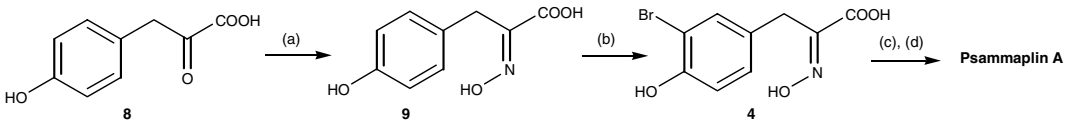
Based on our longstanding interest in DNA methylation inhibitors, we next evaluated psammaplin A and 5-Br-psammaplin A for possible in vitro effects on DNA methyltransferase (DNMT) activity and in cell culture, for cytotoxicity and the ability to reactivate methylation-silenced genes. Psammaplin A was previously reported to inhibit DNMT in vitro.[10] Thus, to confirm the biological activity of the compounds produced by our novel synthetic route, we used a standard assay to examine whether psammaplin A or 5-Br-psammaplin A was able to inhibit DNMT catalyzed by the bacterial methyltransferase SssI.[20] In vitro, both compounds were effective inhibitors of DNMT SssI methyltransferase activity (Fig. 1).



Scheme 1. Hoshino et al. synthesis of psammaplin A.[15] Reagents: (a) TFAA; (b) TFA; (c) NH_2OH; (d) DCC, TEA, NHPHTH, and cystamine.



Scheme 2. Nicolaou et al. synthesis of psammaplin A.[7] Reagents: (a) $KBr/KBrO_3$; (b) TFAA; (c) TFA; (d) NH_2OTHP; (e) NHS, EDC; (f) TEA, cystamine; (g) HCl, MeOH.



Scheme 3. Improved synthesis of psammaplin A. Reagents and conditions: (a) NH_2OH (2 equiv), pyridine, rt, 3 h (79%); (b) KBr (1.5 equiv), $KBrO_3$ (0.3 equiv), 0.25 N aq HCl, rt, 40 min (81%); (c) NHS (1.5 equiv), DCC (1.5 equiv), DMF, rt, 6–8 h; (d) TEA (2 equiv), cystamine (0.5 equiv), rt, 14 h (67%).

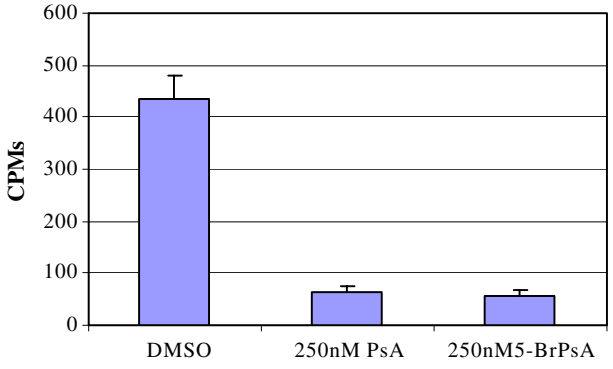


Figure 1. PsA and 5-BrPsA inhibit bacterial Sss1 cytosine DNA methyltransferase activity. Triplicate samples of 0.5 μg poly(dI):dC (Amersham Biosciences, Piscataway, NJ) mixed with the indicated agent along with six units of CpG methylase SssI (New England Biolabs, Beverley, MA) and ^{3}H-SAM (Perkin-Elmer Life Sciences, Boston, MA). After a 2-h incubation at 37 °C, DNA was recovered by phenol extraction and ethanol precipitation. Unincorporated ^{3}H-SAM was removed using the Qiaquick PCR Purification Kit (Qiagen, Valencia, CA). Radiolabeled poly(dI):dC was quantified by scintillation counting.

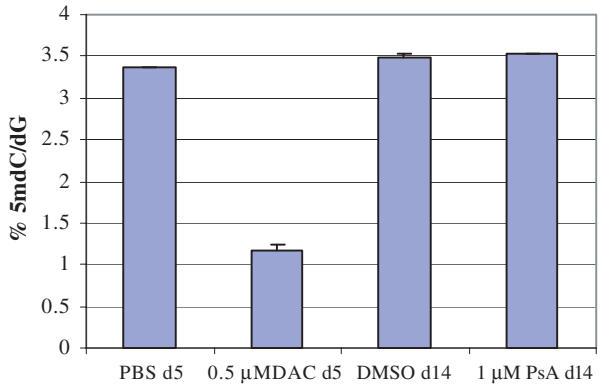


Figure 2. PsA treatment fails to reduce genomic DNA methylation in colon tumor cells. Genomic DNAs were harvested from HCT116 cells treated as indicated, and triplicate samples were hydrolyzed and analyzed by LC–MS to determine 5mdC concentrations.

To further examine the potential activity of psammaplin A as a DNMT inhibitor, we treated cultured HCT116 human colon carcinoma cells with psammaplin A (1 μM, on days 0, 2, 4, 6, 8, and 10) and evaluated global genomic DNA methylation using a mass spectrometry assay.[21] At fourteen days (d14) we found no changes in global 5-methyl-2′-deoxycytidine (5mdC) levels when compared to a control with phosphate-buffered saline (PBS), even after prolonged psammaplin A treatment (Fig. 2). In a parallel experiment (Fig. 2), the nucleoside analog 5-aza-2′-deoxycytidine (DAC, 0.5 μM), an established DNMT inhibitor,[22] effectively reduced 5mdC levels in HCT116 colon carcinoma cells within five days. We also found that psammaplin A treatment of HCT116 cells failed to induce the hypomethylation or reactivation of cancer-testis antigen genes, known methylation-silenced genes (data not shown).[23] Notably however, psammaplin A treatment did cause substantial cellular cytotoxicity in HCT116 cells at low micromolar concentrations (Supplementary Figure 1). In contrast, 5-Br-psammaplin A, evaluated at the same concentrations, was not cytotoxic. Thus, we conclude that DNMT is likely not a major cellular target of psammaplin A. It is possible that psammaplin A is not effectively transported into the nucleus, which is required for DNMT inhibition.

The seemingly distinct mechanisms by which psammaplin A inhibits a diverse group of enzymes are unclear (Table 1). The fact that several of these proteins are key DNA replication enzymes is intriguing, since psammaplin A and DNA do not appear to have common structural features. We anticipate that the improved synthesis and increased availability of psammaplin A will enable its use for X-ray crystallographic studies to determine its mode of binding to these specific proteins.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2006.03.008.

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