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Versatile Routes to Marine Sponge Metabolites through Benzylidene Rhodanines

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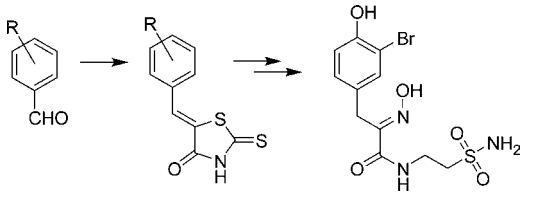
ABSTRACT



The first total synthesis of the marine natural products Psammaplin C and Tokaradine A is described. Benzylidene rhodanines were utilized as versatile intermediates toward the synthesis of seven brominated marine sponge metabolites through the optimization of protection group strategies. Spermatinamine demonstrated good inhibition of all cancer cell lines tested, in particular the leukemia K562 and colon cancer HT29 cell lines.

Over the past 40 years marine sponges of the order Verongida have provided a wealth of brominated compounds with a broad range of biological activities.[1] Some of the most notable tyrosine oxime metabolites include the dimeric Spermatinamine,[2] the sulfur containing dimeric Psammaplin A,[3] Bisaprasin,[4] and the unusual *N*-substituted pyridinium containing Tokaradine A (Figure 1).[5]

General routes to synthesizing α-oximino amide derived natural products from precursor benzaldehydes have included azlactone hydrolysis−oximation, Horner−Wadsworth−Emmons (HWE), and cyano-ylide chemistry.[6] A major drawback to using these methods is the harsh conditions required (TFA, LDA, and O_3 respectively), which requires specific protection−deprotection strategies if the starting substituted benzaldehyde contains sensitive

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groups. In order to reach some of the more sensitive metabolites in larger quantities for biological evaluation, a general method is required which involves stable intermediates.

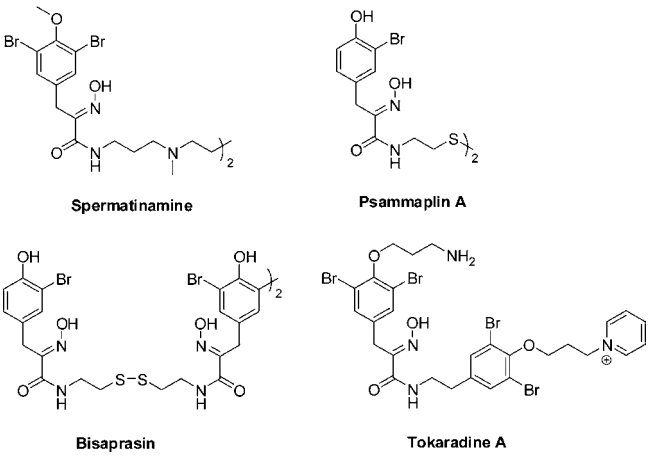
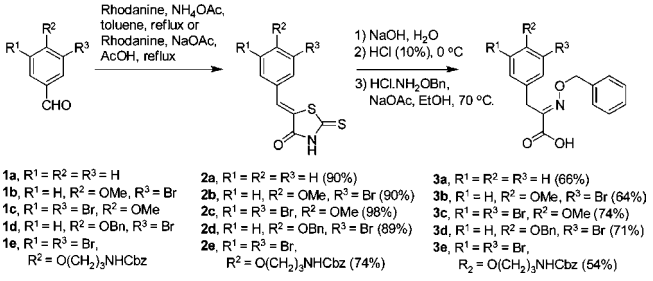


Figure 1. Brominated tyrosine oxime metabolites.

Benzylidene rhodanines are easily prepared through the condensation of an appropriate benzaldehyde with rhodanine and hydrolysis under basic conditions to give the corresponding 3-aryl-mercaptoacrylic acids, which are prone to spontaneous dimerization under oxidative conditions.[7] Oximation of 3-arylmercaptoacrylic acids with hydroxylamine has been shown to proceed in high yields[8] and could thus provide a reliable route to *O*-substituted oximino acids, by analogy to work using arylpyruvic acids.[9] The previous formation of substituted *O*-substituted oximino acids has focused upon *O*-PMB-, *O*-THP-, and *O*-benzyl-substituted hydroxylamines due to ease of synthesis and availability.[10]

Scheme 1. Synthesis of *O*-Benzyl Protected Oximes



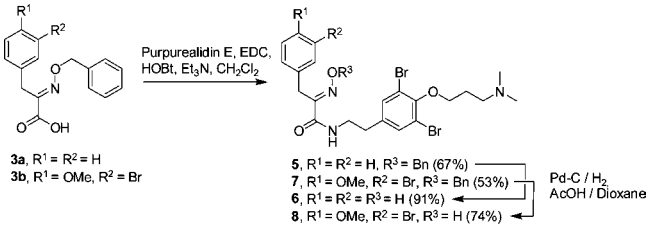
Starting with the appropriate benzaldehyde **1a**–**e** it was envisaged that the corresponding *O*-protected oximino acids **3a**–**e** could be prepared through a two-step procedure and then utilized in a subsequent coupling/deprotection to give the sponge metabolites (Scheme 1).

All benzaldehydes **1a**–**e** were converted to their benzylidene rhodanines **2a**–**e** in good to excellent yield on a multigram scale involving a simple workup.

The three-step conversion of **2a**–**e**, involving hydrolysis, acidification, and subsequent oximation, gave *O*-benzyl protected oximino acids **3a**–**e** on a gram scale, in good yields after chromatography. The intermediate 3-arylmercaptoacrylic acids were not isolated but converted immediately to avoid oxidative dimerization.

20-*N*-Methylpurpuramine E **6** is an analogue of purpuramine E[11] which has been isolated from the Okinawan sponge *Pseudoceratina purpurea* and has weak cytotoxicity (IC_{50} = 4.3 μg mL^{-1}) against HeLa S_3 cells.[12] Aplysamine-2 **8** was isolated from the Australian sponge *Aplysina* sp. and was previously synthesized in our group *via* the amination of the oximino methyl ester. Gram positive bacteria demonstrate moderate susceptibility to aplysamine-2.[13,11a] Carbodiimide coupling of **3a** or **3b** with purpuramine E, followed by benzyl deprotection, provided 20-*N*-methylpurpuramine E **6** (61%, two steps) and aplysamine-2 **8** (38%, 2 steps), the structures of which were confirmed by comparison of their spectra data with those of the natural products (Scheme 2, Tables S1 and S2).

Scheme 2. Synthesis of 20-*N*-Methylpurpuramine E and Aplysamine-2



5-Bromoverongamine **10** was isolated from Caribbean sponge *Pseudoceratina* sp. and reported to inhibit the settlement of barnacle larvae, to be bactericidal toward methicillin resistant *S. aureus* (MRSA) and slightly cytotoxic

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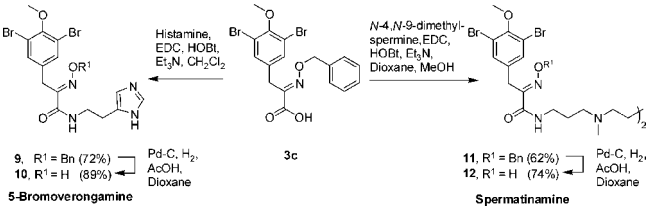
to HeLa cells ($IC_{50} > 50\ \mu g\ mL^{-1}$).[14] Spermatinamine **12** was isolated from *Pseudoceratina* sp. collected in Australia and was shown to be the first natural product inhibitor ($IC_{50} = 1.9\ \mu M$) of isoprenylcysteine carboxyl methyltransferase (Icmt). It has been synthesized *via* oxidation of the tyrosine precursor and azlactone hydrolysis–oximation.[15]

The synthesis of 5-bromoverongamine **10** (64%, two steps, Scheme 3) proceeded from **3c** in a manner analogous to that for the preparation of **7** and **8**. Under similar carbodiimide coupling conditions (amine, EDC, HOBt, Et_3N, CH_2Cl_2) **3c** failed to couple with *N*-4,*N*-9-dimethylspermine,[15c] presumably due to solubility issues. Changing the solvent system to dioxane/methanol (9:1) provided the desired product **11** in good yield, and this was subsequently deprotected to give spermatinamine **12** (46%, two steps) (Scheme 3). The structures of both **10** and **12** were again confirmed through direct comparison with the spectral data for the natural products (Tables TS3 and TS4).

Scheme 3. Synthesis of 5-Bromoverongamine and Spermatinamine



Psammaplin C **14** has been isolated from the sponge *Pseudoceratina purpurea*, but, due to the compound's scarcity, broad biological evaluation has not been determined.[16] In contrast, psammaplin A **16** has been isolated in abundance from a number of sponges, including *Pseudoceratina purpurea*,[3,4,16a,16b,17] and has been shown to inhibit mycothiol-*S*-conjugate amidase,[18] DNA gyrase,[19] topoisomerase II,[20] and histone deacetylases (HDACs) as well as DNA-methyltransferases (DNMTs).[17]

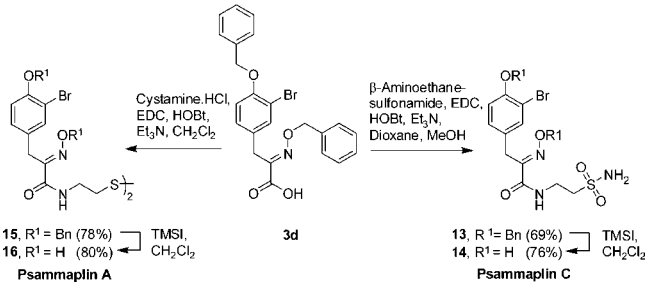
The coupling of **3d** with cystamine and β-aminomethanesulfonamide proceeded in good yield to give the benzyl protected sponge metabolites **15** (78%) and **13** (69%) respectively (Scheme 4). Several attempts at the deprotection of **13** and **15** using hydrogenolysis resulted in no reaction or decomposition, possibly as a result of catalyst poisoning, desulfurization, and disulfide bond breakage. Benzyl group deprotection with $BCl_3 \cdot SMe_2$ in DCM has produced excellent results for similar compounds[21] but, in our hands, resulted in decomposition.

Deprotection of **13** and **15** using TMSI[22] in CH_2Cl_2 proceeded smoothly to provide psammaplin C **14** (76%) and psammaplin A (**16**, 80%), respectively. Once again, the structures of both **14** and **16** were confirmed by direct comparison with the spectral data for the natural products (Tables TS5 and TS6).

Tokaradine A **20** was isolated from *Pseudoceratina purpurea* collected in Japan and found to be lethal to the crab *Hemigrapsus sanguineus*.[5]

The synthesis of tokaradine A (Scheme 5) proceeded through the preparation of amine **18** as the ditriflate salt from the alkylation of *N*-Boc-3,5-dibromo-4-hydroxyphenethylamine[23] with 1-(3-bromopropyl)pyridinium bromide,[24] to give **17** (87%). Boc deprotection to give **18** (97%), followed by carbodiimide coupling with acid **3e**, provided the Cbz-benzyl protected derivative **19** (71%) in good yield after chromatography.

Scheme 4. Synthesis of Psammaplin C and Psammaplin A



Hydrogenation of **19** under the standard conditions used for the preparation of **5**, **6**, **9**, and **11** proved unsuccessful, resulting in complex mixtures of *O*- and *N*-deprotection and decomposition. Deprotection using TMSI in CH_2Cl_2 (12 h at 40 °C or rt up to 14 days) also resulted in mixtures of deprotected (*O*-, *N*-) oxime reduced material and trace amounts of **20** (detected by MALDI-MS).

The use of $AlCl_3$ and anisole in CH_2Cl_2/CH_3NO_2 has been reported for the removal of *N*-Cbz and *O*-PMB protecting groups from oxime-containing bromotyrosines.[11b] In our hands these conditions, at rt for 48 h, yielded a mixture of **20**, the *O*-Bn analogue, and oxime reduced products which proved difficult to separate through column chromatography. These results suggest the use of either $AlCl_3$ or

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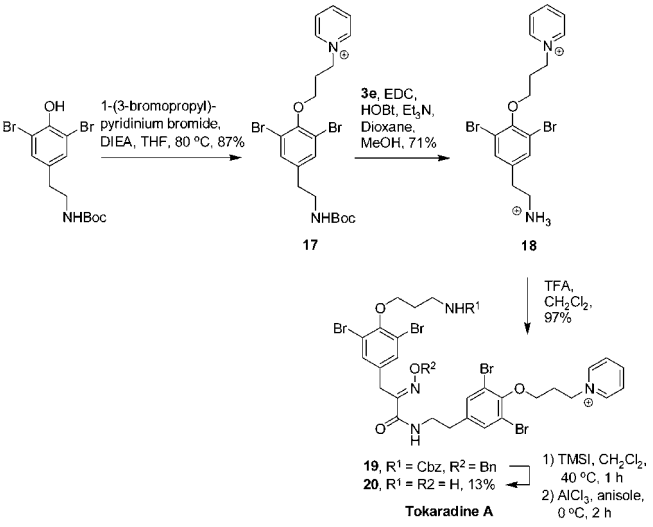
Scheme 5. Synthesis of Tokaradine A



TMSI does not afford complete deprotection, promoting *N*-deprotection and *O*-deprotection/reduction respectively. TMSI and $AlCl_3$/anisole together have been reported to remove *N*-Cbz and *O*-Bn protecting groups successfully from macrolide antibiotics,[25] and a two-step, one-pot procedure provided tokaradine A **20** (13%) after column chromatography. Direct comparison with the spectral data of the extracted natural product confirmed the structure (Table TS7).

All synthesized natural products (**6**, **8**, **10**, **12**, **14**, **16**, **20**) were assessed for their antibacterial and antifungal properties in accordance with the recommendations of the British Society of Antimicrobial Chemotherapy (Table TS8).[26] Apart from psammaplin C **14**, all the other natural products demonstrated medium to good activity against Gram positive bacteria, most noteworthy tokaradine A (**20**, MIC = 8 μg/mL against *S. epidermidis*, *S. aureus*, and MRSA), 5-bromoverongamine **10** (MIC $\leq$ 8 μg/mL against *S. pyogenes*), and psammaplin A **16** (MIC $\leq$ 1 μg/mL against *S. epidermidis*). Only **6**, **10**, and **20** demonstrated weak activity against Gram negative bacteria. MICs against mycobacteria were determined for **6**, **8**, **10**, **16**, and **20** using a spot culture growth inhibition assay (SPOTi),[27] which has been previously used to evaluate antitubercular natural products[28] and novel synthetic compounds.[29] 20-*N*-Methylpurpuramine E **6** and tokaradine A **20** showed potent growth activity against *M. bovis* BCG (MIC = 5 μg/mL) and *M. tuberculosis* $H^{37}Rv$ (MIC = 16 μg/mL) respectively. Only **20** displayed weak antifungal activity against two pathogenic strains tested.

Due to its promising biological activity demonstrated in the initial one-dose screen against the NCI 60 human tumor cell line panel, and as a known Icmt inhibitor, spermatinamine **10** was selected for rescreening by the Biological Evaluation Committee over the ranges given in Table TS15. Spermatinamine inhibited all cell lines at single-digit micromolar concentrations (average GI_{50} = 0.23 μM) or below (Leukemia K562 GI_{50} = 0.93 μM, Colon cancer HT29 GI_{50} = 0.59 μM).

In summary, this paper describes the first syntheses of the marine sponge secondary metabolites psammaplin C **14** and tokaradine A **20** and a robust synthetic route to a range of brominated marine sponge metabolites, including the more problematic sulfur containing analogues. Spermatinamine **10** showed promising results against the NCI 60 cell line cancer screen, and we are currently exploiting the versatility of our synthetic strategy to produce a range of analogues of the compounds reported here in order to obtain SAR data.

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Supporting Information Available. Synthetic procedures, analytical and biological data for compounds **2a**–**e**, **3a**–**e**, **5**–**20**. This material is available free of charge via the Internet at http://pubs.acs.org.

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The authors declare no competing financial interest.