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Total Synthesis of Psammaplysenes A and B, Naturally Occurring Inhibitors of FOXO1a Nuclear Export

Savvas N. Georgiades and Jon Clardy*

Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, Massachusetts 02115

jon_clardy@hms.harvard.edu

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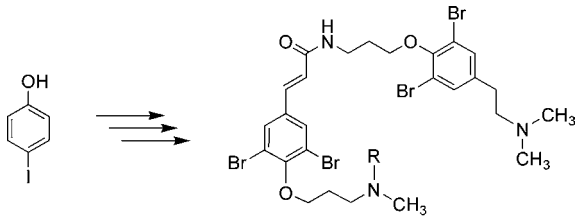
ABSTRACT



Psammaplysene A: **R=CH$_3$**
Psammaplysene B: **R=H**

Two inhibitors of FOXO1a-mediated nuclear export, psammaplysenes A and B, have been synthesized by a flexible and efficient route. A common starting material, 4-iodophenol, was used to prepare both halves of these pseudosymmetric dibromotyrosine-derived metabolites.

A balance between promoting and inhibitory signals governs cell growth, and dysregulation of this balance can result in disease. In a specific example, the growth-promoting PI3-kinase signal is countered by the growth-inhibiting PTEN-phosphatase signal. Thus, loss of function mutations in PTEN can result in an inappropriate increase in stimulatory signals, and such mutations have been linked with Cowden's disease, a hereditary disease with a predisposition to breast and thyroid cancer, as well as other cancers.[1]

Finding a small molecule that could directly compensate for a loss of function mutation in PTEN was deemed unlikely, so a set of screens to identify small molecules that could indirectly compensate for the loss of PTEN activity by modulating some downstream target was designed.[2] One consequence of loss of PTEN-phosphatase activity is the export of the Forkhead transcription factor FOXO1a from the nucleus to the cytoplasm. In the nucleus, FOXO1a can function as a transcriptional regulator of apoptosis or cell cycle arrest to counteract inappropriate PI3-kinase generated signals, but its transport to the cytoplasm abolishes that regulatory activity. In a high-content screen to identify small molecules able to enforce the nuclear localization of FOXO1a, psammaplysene A (**1**) was identified as one of the most potent of the pathway-specific screening positives.[2,3] Psammaplysene B (**2**) was somewhat less potent. Since the supply of these dibromotyrosine-derived metabolites which came from an Indian Ocean marine sponge (*Psammaplysilla* sp.) was very limited, a total synthesis was developed. The synthetic route was designed to be flexible in order to additionally prepare focused libraries for further testing or probes to identify the still unknown target of **1** and **2**.

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(3) Schroeder, F. C.; Kau, T. R.; Silver, P. A.; Clardy, J. *J. Nat. Prod.* **2005**, *68*, 574.

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The *Psammaplysilla* sp. sponge from which **1** and **2** were isolated belongs to a family associated with many tyrosine-derived metabolites with a range of biological activities. For example, purpuramine I has antimicrobial properties,[4] psammaplin A is both a histone deacetylase inhibitor and a DNA methyltransferase inhibitor,[5] and bastadin 5 is a potent agonist of the RyR1 calcium channel.[6] Unlike these compounds, **1** and **2** have a head-to-tail connectivity between the two dibromotyrosine subunits and an α,β-unsaturated amide linkage that are unusual features in this family of natural products.

A retrosynthetic disconnection at the amide bond of psammaplysenes (Figure 1) yields two similar fragments, **3**

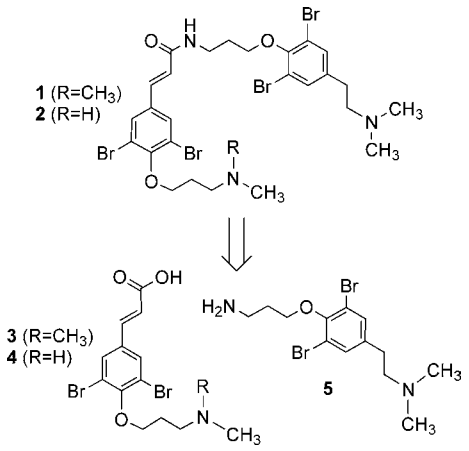
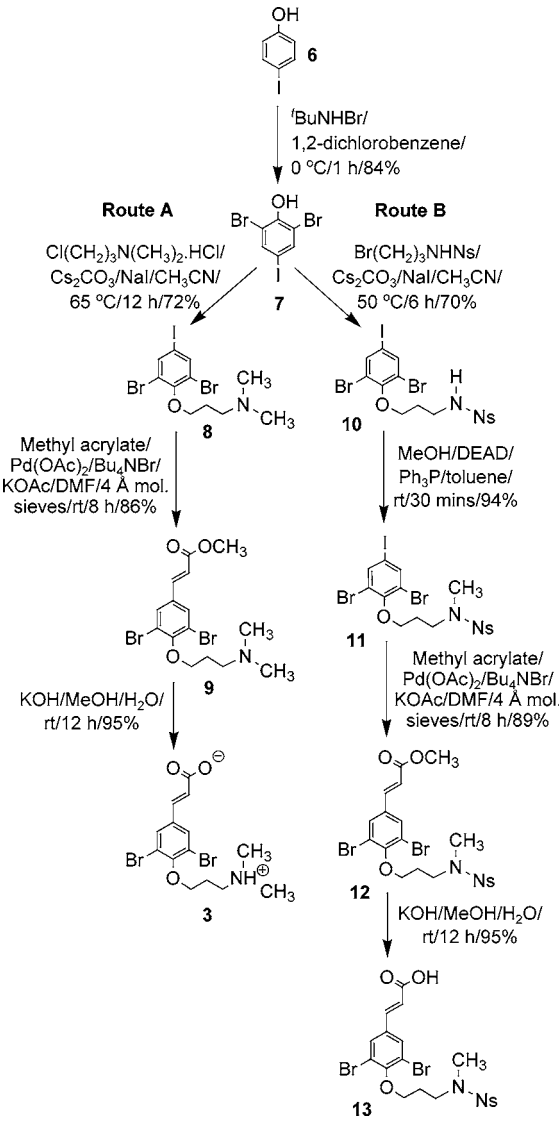


Figure 1. Structures of psammaplysene A (**1**) and B (**2**) and main retrosynthetic disconnection.

(or **4**) and **5**. Both of these fragments comprise a dibromophenol ring with the oxygen attached to a three-carbon linker with a terminal amine. They differ by the sp^2-sp^2 C−C bond linking the ring's para position to the α,β-unsaturated carbonyl moiety in **3** (or **4**) vs the sp^2-sp^3 C−C bond between the ring's para position and the two-carbon tail with the terminal tertiary amine in **5**. Both halves undoubtedly originate in a common tyrosine precursor, and following this logic, our approach began with 2,6-dibromo-4-iodophenol (**7**).

Precursor **7** (84% yield) was prepared via directed ortho-bromination of 4-iodophenol (**6**) with *N*-bromo-*tert*-butylamine,[7,8] which was superior to other brominating agents, such as Br_2/KBr and NBS, in avoiding iodine−bromine exchange in the para position. Compound **7** was transformed

Scheme 1



to **3** in three steps (Scheme 1, route A). The *O*-alkylation of **7** to **8** (72% yield) with 1-chloro-3-dimethylaminopropane hydrochloride required heating in CH_3CN, in the presence of Cs_2CO_3 and catalytic NaI. A Pd^{2+}-catalyzed cross-coupling of iodide **8** to methyl acrylate gave methyl ester **9** (86% yield). A modification of Heck reaction conditions described by Jeffery,[9] involving the use of $Pd(OAc)_2$, KOAc, and Bu_4NBr, proved to be compatible with the tertiary amine in this transformation. Saponification of methyl ester **9** in MeOH−H_2O with excess KOH for 12 h yielded acid **3** (95%) which, based on ^1H NMR chemical shifts,[10] exists in zwitterionic form.

A suitable protecting group, 2-nitrobenzenesulfonyl (Ns), was required for the preparation of **2**. Its activating effect

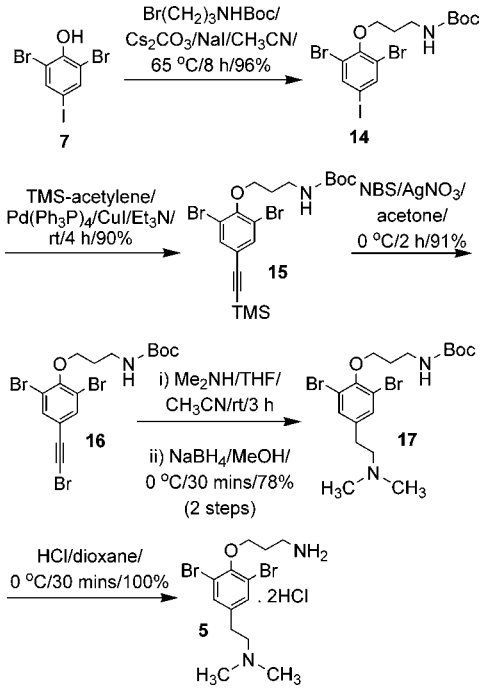
(4) Yagi, H.; Matsunaga, S.; Fusetani, N. *Tetrahedron* **1993**, *49*, 3749.
(5) Piña, I. C.; Gautschi, J. T.; Wang, G. Y. S.; Sanders, M. L.; Schmitz, F. J.; France, D.; Cornell-Kennon, S.; Sambucetti, L. C.; Remiszewski, S. W.; Perez, L. B.; Bair, K. W.; Crews, P. *J. Org. Chem.* **2003**, *68*, 3866.
(6) Mack, M. M.; Molinski, T. F.; Buck, E. D.; Pessah, I. N. *J. Biol. Chem.* **1994**, *269*, 23236.
(7) *N*-Bromo-*tert*-butylamine was prepared following a published method: Boozer, C. E.; Moncrief, J. W. *J. Org. Chem.* **1962**, *27*, 623.
(8) For ortho-directed bromination of unsubstituted phenol with *N*-bromo-*tert*-butylamine, see: Jpn. Kokai Tokkyo Koho, 09291053, 11 Nov 1997, Heisei.
(9) Jeffery, T. *Tetrahedron* **1996**, *52*, 10113.
(10) Significant shifts downfield for the peaks corresponding to the methyl and methylene protons on carbons directly attached to the nitrogen of **3** suggest that the tertiary amine is protonated; therefore, **3** must be overall a zwitterion given that it was prepared and isolated in nonacidic conditions. Considerable pH-dependent shifts, even for protons which are two carbons away from the nitrogen, are not unusual for such basic systems.

on the nucleophilicity of primary sulfonamides[11] allowed us to start with a Ns-protected primary amine and later convert it to the desired Ns-protected secondary amine. Alkylation of **7** with Ns-protected 3-bromopropylamine[12] afforded **10** (70% yield, Scheme 1, route B). Adding a methyl to the sulfonamide nitrogen of **10** was achieved by using Fukuyama's method,[13] methanol functionalization with triphenylphosphine and diethyl azodicarboxylate in toluene,[14] to give **11** (94% yield). A Heck reaction produced methyl ester **12** (89% yield) from **11**. Finally, hydrolysis of **12** provided acid **13** (95% yield).

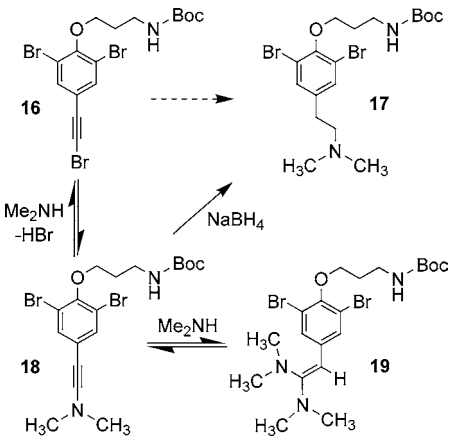
The common part of both psammaplysenes, **5**, was prepared as shown in Scheme 2. *O*-Alkylation of **7** with Boc-protected 3-bromopropylamine[15] afforded **14** (96% yield). Our initial attempts to cross-couple iodide **14** to an appropriate organometallic reagent to directly introduce a saturated two-carbon piece bearing a terminal masked aldehyde were unsuccessful.[16] The conventional Friedel−Crafts approach was also abandoned due to the disappointing conversions we observed with a similar aromatic system lacking the 4-iodo substituent.[17] The ring's apparently unusual reactivity led us to investigate an alternative for converting **14** to **5**. Using catalytic amounts of $Pd(Ph_3P)_4$ and CuI in triethylamine (Sonogashira conditions[18]), iodide **14** was readily cross-coupled to ethynyltrimethylsilane to afford alkyne **15** (90% yield). The TMS-protected alkyne was subsequently converted to alkynyl bromide **16** (91% yield) via a silver-assisted desilylative bromination with NBS in acetone.[19] Subsequent aminolysis of bromide **16** using 10-fold excess of dimethylamine in THF−CH_3CN followed by in situ reduction of the intermediate with 1 M $NaBH_4$ in MeOH provided tertiary amine **17** (78% yield). The presence of acetonitrile in the reaction mixture seemed to enhance the reaction rate and minimize formation of undesired products. Boc-protected amine **17** was converted to the dihydrochloride salt of primary amine **5** upon treatment with excess 4 M HCl in dioxane.[20] LC−MS indicated a quantitative deprotection after 30 min at 0 °C.

The two-step transformation of **16** to **17** presumably proceeds via an unusual ynamine intermediate (**18**, Scheme 3), which is quite different from the electron-deficient ynamines previously reported to be synthetically useful.[21] Even though this elusive ynamine species was not directly detected, an adduct heavier than **18** by one additional dimethylamine unit was observed by LC−MS as the major aminolysis product. An aliquot obtained from the reaction mixture after full consumption of **16** was chromatographi-

Scheme 2



Scheme 3



(11) (a) Kan, T.; Fukuyama, T. *Chem. Commun.* **2004**, *4*, 353. (b) Kan, T.; Fukuyama, T. *J. Synth. Org. Chem. Jpn.* **2001**, *59*, 779.

(12) For preparation, see the Supporting Information.

(13) (a) Fukuyama, T.; Jow, C. K.; Cheung, M. *Tetrahedron Lett.* **1995**, *36*, 6373. (b) Fukuyama, T.; Cheung, M.; Jow, C. K.; Hidai, Y.; Kan, T. *Tetrahedron Lett.* **1997**, *38*, 5831.

(14) Mitsunobu, O. *Synthesis* **1981**, *1*, 1.

(15) For preparation, see the Supporting Information.

(16) Allyl-Grignard and (1,3-dioxolan-2-ylmethyl)-Grignard reagent were screened with catalysts $NiCl_2$(dppp), $Fe(TMHD)_3$, and $PdCl_2$(dppf) under various conditions. Analogous organozinc reagents were screened with catalysts $Pd(Ph_3P)_4$, $PdCl_2[(o\text{-tolyl})_3P]_2$, and $PdCl_2$(dppf). All cases suffered from either unacceptable yields or poor regioselectivity for the *p*-iodide position vs the *o*-bromide positions.

(17) 2-Chloroacetyl-chloride was tried with catalyst $AlCl_3$ in different solvents, under various conditions.

(18) Sonogashira, K.; Tohda, Y.; Hagihara, N. *Tetrahedron Lett.* **1975**, *16*, 4467.

(19) (a) Bowles, D. M.; Anthony, J. E. *Org. Lett.* **2000**, *2*, 85. (b) Tobe, Y.; Nakagawa, N.; Kishi, J. Y.; Sonoda, M.; Naemura, K.; Wakabayashi, T.; Shida, T.; Achiba, Y. *Tetrahedron* **2001**, *57*, 3629. (c) Tobe, Y.; Utsumi, N.; Nagano, A.; Sonoda, M.; Naemura, K. *Tetrahedron* **2001**, *57*, 8075.

(20) Han, G.; Tamaki, M.; Hruby, V. J. *J. Peptide Res.* **2001**, *58*, 338.

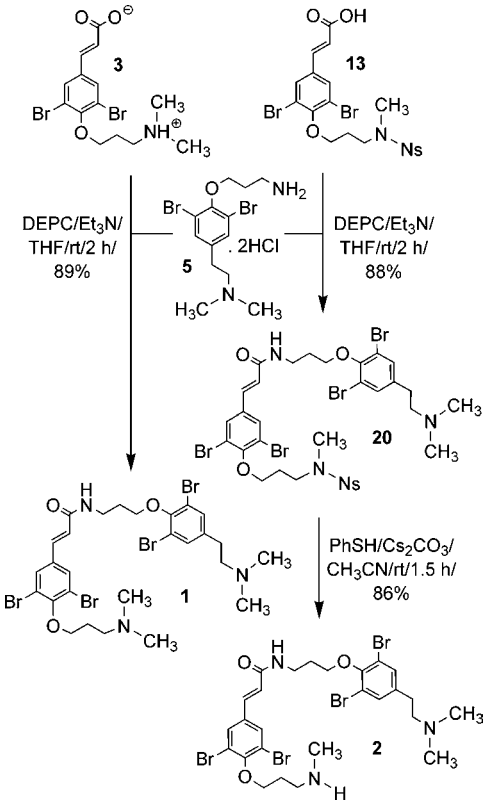
(21) For a recent review on ynamine and ynamide research, see: Zificsak, C. A.; Mulder, J. A.; Hsung, R. P.; Rameshkumar, C.; Wei, L. L. *Tetrahedron* **2001**, *57*, 7575 and references therein.

cally fractionated to provide a small amount of the adduct, for which ^{1}H NMR suggested structure **19**.[22] Masked ynamines resulting from addition of a second amine molecule to a preformed ynamine have been previously reported in a few cases.[23] Despite the relative stability of **19** in both the strongly basic aminolysis environment and during purification, its formation was reversed under $NaBH_4$-induced reducing conditions. This spontaneous process most likely occurs via elimination of dimethylamine and stepwise reduction following a mechanism similar to the usual reduction of enamines.

The final amide coupling steps leading to the assembly of the complete psammaplysenes skeleton were carried out by using diethylphosphocyanidate, under basic conditions established by triethylamine in THF (Scheme 4). This transformation generated psammaplysene A (**1**, 89% yield) and the Ns-protected psammaplysene B (**20**, 88% yield). Removal of the Ns group of **20** to give psammaplysene B (**2**, 86% yield) was accomplished with thiophenol and Cs_2CO_3 in CH_3CN, taking advantage of the susceptibility of the nitro-activated ring to aromatic nucleophilic substitution on the 1-position.[11,13] The synthetic samples of **1** and **2** exhibited the same spectroscopic profiles and biological properties as the corresponding natural products.

In summary, efficient routes to psammaplysenes A (**1**) and B (**2**) were developed. The two major fragments were assembled from the same starting material, and a method for constructing the phenethylamine system through initial introduction of an alkyne was developed. The overall scheme should be useful in preparing a focused library of psammaplysene analogues.

Scheme 4



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Supporting Information Available: Experimental procedures and spectroscopic data. This material is available free of charge via the Internet at http://pubs.acs.org.

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(22) ^{1}H NMR of **19** in $CDCl_3$ showed all 12 methyl protons as equivalent at 3.37 ppm and one proton at 4.44 ppm, in good correlation with predictions for the methyl and vinylic protons of the proposed 2,2-bis(dimethylamino)-vinyl system. Moreover, the vinylic proton was exchanged with deuterium in CD_3OD, whereas the rest of the peaks remained essentially unaffected.

(23) A recent example involved formation of bis-pyrrole adducts upon treatment of alkylthiochloroacetylenes with pyrrole: Sobenina, L. N.; Dyachkova, S. G.; Stepanova, Z. V.; Toryashinova, D. S. D.; Albanov, A. I.; Ushakov, I. A.; Demenev, A. P.; Mikhaleva, A. I.; Trofimov, B. A. *Russ. J. Org. Chem.* **1999**, *35*, 916.