ORGANIC LETTERS

2006
Vol. 8, No. 2
321−324

Liphagal, a Selective Inhibitor of PI3 Kinase α Isolated from the Sponge *Aka coralliphaga*: Structure Elucidation and Biomimetic Synthesis

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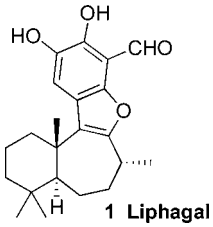
Received November 12, 2005

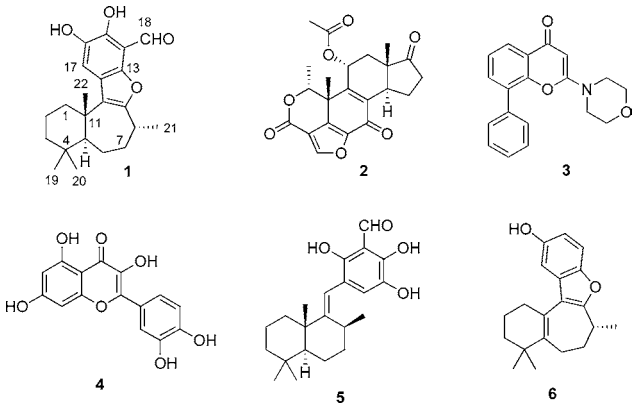
ABSTRACT



1 Liphagal

Liphagal (1), a selective inhibitor of PI3K α, has been isolated from the marine sponge *Aka coralliphaga* collected in Dominica. The "liphagane" meroterpenoid carbon skeleton of liphagal (1) is new. A biomimetic total synthesis has been used to confirm the constitution of liphagal (1) and support a proposed biogenesis.

The phosphatidylinositol-3-kinase (PI3K) signaling pathway plays a central role in regulating cell proliferation, cell survival, adhesion, movement, differentiation, membrane trafficking, glucose transport, neurite outgrowth, and superoxide production.[1−3] A key second messenger in this pathway is phosphatidylinostitol-3,4,5-trisphosphate (PI-3,4,5-P_3), which is present at low concentrations in unstimulated cells but is rapidly synthesized from PI-4,5-P_2 by PI3K in response to a wide array of extracellular stimuli. To tightly regulate PI3K signaling, cells degrade PI-3,4,5-P_3 to PI-4,5-P_2 with the tumor suppressor PTEN and to PI-3,4-P_2 with SHIP, sSHIP, or SHIP2.[4]



There are several closely related PI3K isoforms that are thought to have different biological activities. Isoform

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10.1021/ol052744t CCC: $33.50
Published on Web 12/18/2005

selective drugs capable of attenuating PI3K signaling should have significant therapeutic potential for the treatment of inflammatory and autoimmune disorders as well as cancer and cardiovascular diseases.[1−4] Wortmannin (**2**) and LY294002 (**3**), a synthetic analogue of the flavanoid quercetin (**4**), are two first-generation PI3K inhibitors that have been widely employed as chemical genetics probes to elucidate the biological roles of PI3K signaling. Second-generation isoform-selective PI3K inhibitors, whose structures appear to be based on quercetin and LY294002, have been described in the patent literature.[5−7] It has also been reported that AS-605240 and related thiazolidine-2,4-diones are selective submicromolar inhibitors of PI3K γ.[8]

We have screened a library of marine invertebrate extracts in a fluorescent polarization assay using human PI3K α expressed in SF9 insect cells as part of a program designed to find new isoform-selective PI3K inhibitors. The MeOH extract of the sponge *Aka coralliphaga* collected in Dominica showed promising activity in the assay. Bioassay-guided fractionation of the extract identified liphagal (**1**), a meroterpenoid with an unprecedented carbon skeleton, as the only PI3K inhibitory component. The details of the structure elucidation, proposed biogenesis, biomimetic synthesis, and biological activity of liphagal (**1**) are presented below.

Specimens of *A. coralliphaga* were harvested by hand using scuba on reefs in Prince Rupert Bay, Portsmouth, Dominica. Freshly collected sponge was frozen on site and transported to Vancouver over dry ice. Thawed sponge was extracted exhaustively with MeOH, and the combined MeOH extracts were concentrated in vacuo to give an aqueous suspension that was partitioned between H_2O and EtOAc. The H_2O-soluble material, which showed strong PI3K inhibition, was partitioned between BuOH and H_2O. Fractionation of the enzyme-inhibitory BuOH-soluble portion by sequential application of Sephadex LH20 chromatography (eluent: MeOH) and reversed-phase HPLC (eluent: 17:3 MeOH/H_2O) gave a pure sample of liphagal (**1**).

Liphagal (**1**) was obtained as an optically active ($[\alpha]^{25}_D$ +12.0 (*c* 3.7, MeOH)) amorphous yellow solid that gave a $[M]^+$ ion in the HREIMS at *m/z* 356.1994 appropriate for a molecular formula of $C_{22}H_{28}O_4$ (calcd 356.1988), requiring nine sites of unsaturation. The ^{13}C NMR spectrum (Supporting Information) obtained for **1** contained resonances that could be assigned to 22 carbon atoms consistent with the HRMS data. HMQC data showed that only 26 of the hydrogen atoms were attached to carbon (4 × CH_3, 5 × CH_2, 4 × CH, 9 × C), requiring the presence of 2 OHs. Broad singlets at δ 9.29 and 10.28 in the ^1H NMR spectrum, that were not correlated to carbon resonances in the HMQC spectrum, were assigned to the OH protons.

Preliminary analysis of the ^1H and ^{13}C NMR spectra (Table 1, Supporting Information) revealed that liphagal (**1**) had both substituted benzene and terpenoid fragments. One aliphatic methyl doublet (δ 1.36, J = 7.1 Hz, Me-21) and three aliphatic methyl singlets (δ 0.91, Me-19; 0.94, Me-20; 1.28, Me-22) were present in the ^1H NMR spectrum. Resonances that could be assigned to a pentasubstituted benzene ring (δ 107.9, C-14; 114.9, C-17; 119.2, C-12; 140.8, C-16; 145.8, C-13; 147.2, C-15), a tetrasubstituted olefin (δ 124.4, C-10; 155.2, C-9), and a conjugated aldehyde (δ 189.7, C-18) were observed in the ^{13}C NMR spectrum. The benzene, olefin, and aldehyde functionalities accounted for six of the nine required sites of unsaturation, and the remaining three sites had to be rings. Structural features of **1** identified from the NMR data were similar to the structural components of siphonodictyal B (**5**),[9] previously isolated from specimens of *A. coralliphaga* collected in Belize, suggesting that the compounds were related.

A proton resonance at δ 7.43 (H-17) showed strong HMBC correlations to carbon resonances at δ 140.8 (C-16), 147.2 (C-15), and 145.8 (C-13) and a weak correlation (4 bond) to 107.9 (C-14), and the aldehyde proton resonance at δ 10.40 (H-18) showed correlations to carbon resonances at δ 107.9 (C-14), 147.2 (C-15), and 145.8 (C-13). This set of HMBC correlations supported the presence of a pentasubsituted benzene ring in **1** having the same substitution pattern found in the aromatic ring of siphonodictyal B (**5**). COSY correlations identified the spin system extending from the methylene protons at C-1 (δ 1.38 and 2.47) through to the methylene protons at C-3 (δ 1.21 and 1.43), and starting from H-5 (δ 1.50) through to H-8 (δ 3.16) and Me-21 (δ 1.36). HMBC correlations observed between proton resonances at δ 0.91 (Me-19), 0.94 (Me-20), and 1.28 (Me-22) and a carbon resonance at δ 53.4 (C-5); between the proton resonances at δ 0.91 (Me-19) and 0.94 (Me-20) and the carbon resonance at δ 41.3 (C-3); between proton resonances at δ 1.50 (H-5) and 1.78 (H-6$_\alpha$) and a carbon resonance at 38.9 (C-11); between a proton resonance at δ 1.50 (H-5) and carbon resonances at δ 39.7 (C-1) and 19.9 (C-22); and between the proton resonance at δ 1.52 (H-6$_\beta$) and the carbon resonance at δ 34.4 (C-4) confirmed the presence of a six-membered A ring with dimethyl substitution at C-4 and monomethyl substitution at C-11.

The H-8 (δ 3.16) and Me-22 (1.28) resonances showed HMBC correlations to the C-10 (δ 124.4) resonance, and the H-7$_\alpha$ (δ 2.12), H-8 (3.16), and Me-21 (1.36) resonances showed correlations to the C-9 (δ 155.2) resonance, demonstrating the presence of a seven-membered ring B with a tetrasubstituted $\Delta^{9,10}$ olefin. An HMBC correlation observed between the H-17 (δ 7.43) and C-10 (δ 124.4) resonances indicated that the benzene ring carbon C-12 was bonded to C-10. Connecting the oxygenated benzene carbon C-13 (δ

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145.8) and the olefinic carbon C-9 (δ 155.2) through an ether linkage to give a furan completed the final ring required by the unsaturation number of liphagal (**1**) and generated a constitution that was consistent with all of the spectroscopic data.

1D NOESY data provided evidence for the relative stereochemistry of liphagal (**1**). Correlations observed between Me-22 (δ 1.28) and H-1_{eq} (2.47), H-2_{ax} (1.68), Me-19 (0.91), and H-6_{β} (1.52) when Me-22 was irradiated; between Me-21 (δ 1.36) and H-5 (δ 1.50) when Me-21 was irradiated; and between H-3_{ax} (δ 1.21) and both H-1_{ax} (δ 1.38) and H-5 (δ 1.50) when H-3_{ax} was irradiated were only possible if ring A adopted a chair conformation, the 6,7 ring junction was *trans*, and Me-21 was α as shown.

Liphagal (**1**) inhibited PI3K α with an IC_{50} of 100 nM in the primary fluorescent polarization enzyme assay. In the same assay, wortmannin (**2**) had an IC_{50} of 12 nM and LY294002 (**3**) had an IC_{50} of 550 nM. Liphagal (**1**) was approximately 10-fold more potent against PI3K α than against PI3K γ. Secondary in vitro cell assays showed that liphagal (**1**) was cytotoxic to LoVo (human colon: IC_{50} 0.58 μM), CaCo (human colon: IC_{50} 0.67 μM), and MDA-468 (human breast: IC_{50} 1.58 μM) tumor cell lines.

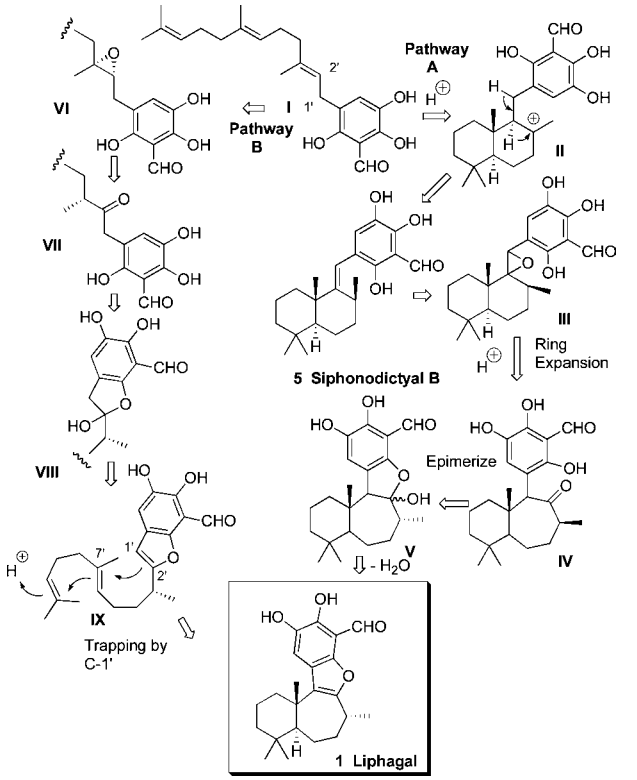
Liphagal (**1**) represents the first example of the "liphagane" meroterpenoid carbon skeleton. Scheme 1 shows two possible biogenetic routes to liphagal. Pathway A involves a proton initiated polyene cyclization of farnesylated trihydroxybenzaldehyde **I** with trapping of the intermediate carbocation by the $\Delta^{2'3'}$ olefin to give **II**, a putative intermediate in the biogenesis of siphonodictyal B (**5**). Siphonodictyal B (**5**) could be converted to liphagal (**1**) via the epoxide **III** followed by ring expansion to ketone **IV**, epimerization at C-8 and hemiketal formation to give **V**, and dehydration. In pathway A, it is the 2′ carbon of the prenyl residue that acts as a nucleophilic center in the polyene cyclization step.

Pathway B outlines a more direct route to liphagal (**1**) that starts with the conversion of **I** to the ketone **VII**, perhaps via the epoxide **VI**. The ketone **VII** should spontaneously form the hemiketal **VIII**, which upon dehydration yields the benzofuran **IX**. Proton-initiated cyclization of **IX** leads directly to liphagal (**1**). Pathway B suggests that the preorganization of a benzofuran fragment represents a way to create nucleophilicity at the 1′ carbon of the prenyl side chain (see **IX**, Scheme 1), thereby committing the polyene cyclization to direct formation of the 6,7 ring system.

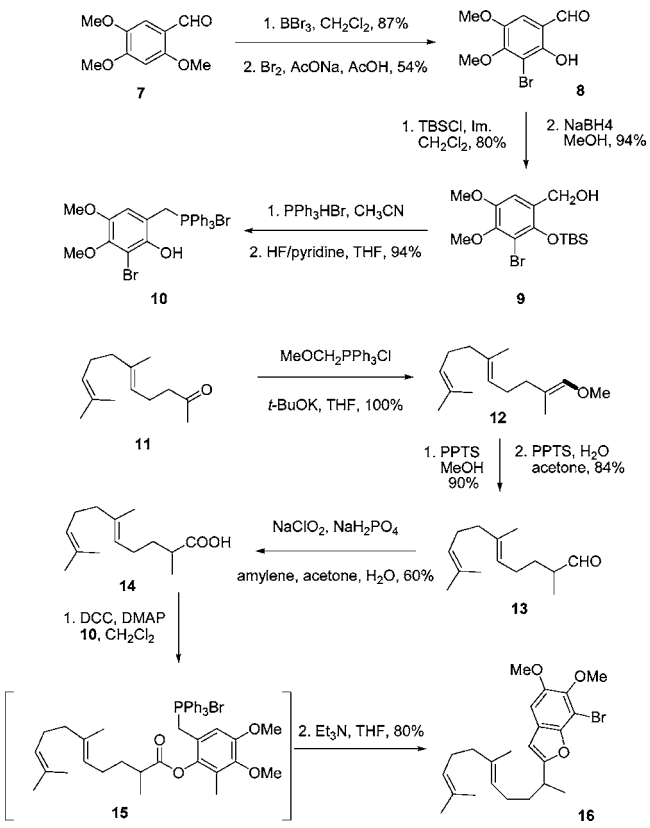
There has been significant interest in the synthesis of frondosin B (**6**).[10−12] The methodologies used to construct the fused 6,7 ring system in frondosin B (**6**) were not readily applicable to the synthesis of liphagal (**1**). We were intrigued by the possibility that cation-initiated cyclization reactions involving trapping by a benzofuran as shown in biogenetic pathway B (Scheme 1) might represent an efficient synthetic approach to liphagal (**1**).

The biomimetic synthesis of racemic liphagal (**1**) started with preparation of the desired cyclization precursor **16** (Scheme 2). Commercially available 2,4,5-trimethoxybenzaldehyde **7** was selectively demethylated at the 2 position

Scheme 1. Proposed Biogenetic Pathways to Liphagal (**1**)



Scheme 2. Synthesis of the Biomimetic Cyclization Precursor **16**



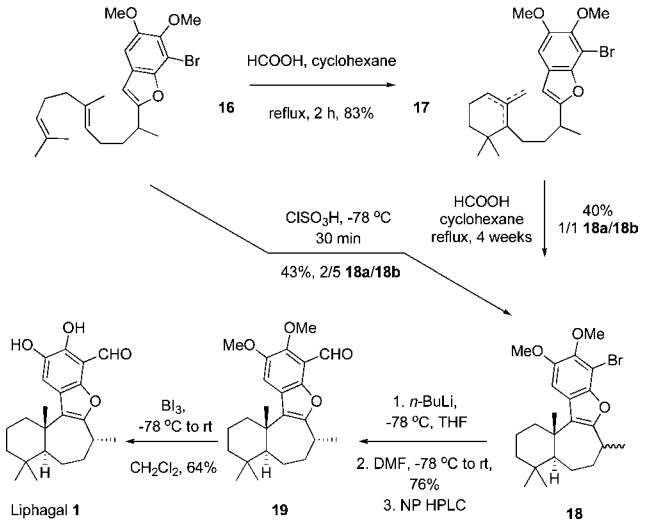
with BBr_3, and the resulting phenol was brominated at C-3 to give **8**. Direct reduction of the aldehyde **8** led to an unstable diol,[13] so it was necessary to use a protection sequence to get the desired phosphonium salt **10**. Protection of the phenol in **8** with TBS, followed by reduction of the aldehyde with sodium borohydride, produced the benzyl alcohol **9**. Treatment of **9** with triphenylphosphine/HBr followed by removal of the TBS protecting group with HF/pyridine complex in THF gave the phosphonium salt **10**.

Synthesis of the isoprenoid fragment started with a Wittig reaction on geranylacetone **11** to give the enol ether **12** (Scheme 2). Direct hydrolysis of the enol ether did not proceed cleanly, so a two-step hydrolysis, first with PPTS and MeOH to give the dimethoxy acetal and then with PPTS in a mixture of acetone and H_2O, was used to generate the aldehyde **13**. Oxidation with $NaClO_2$ converted the aldehyde **13** into the corresponding acid **14**. Coupling the phenol **10** with the acid **14** using DCC gave the ester **15**,[13] which was not isolated. Deprotonation of the phosphonium salt **15** with Et_3N in refluxing THF brought about an intramolecular Wittig reaction resulting in the formation of the desired benzofuran **16**.[14]

The key cyclization step was first effected by refluxing **16** in a biphasic system of formic acid and cyclohexane (Scheme 3)[15] for 14 days to give a racemic mixture **18** of C-8 epimers as a minor product accompanying a mixture of inseparable partially cyclized alkenes **17**, suggesting that the conversion of **16** to **18** proceeded in two steps. In support of this suggestion, it was found that upon treatment with formic acid in refluxing cyclohexane, compound **16** gave within 2 h only a complex mixture of cyclohexenes **17**. The alkenes **17** reacted slowly under the same conditions to give after one month the tetracyclic system **18** in 40% isolated yield as a 1/1 mixture of C-8 epimers **18a** (Me-21α) and **18b** (Me-21β). To shorten the time required for the polyene cyclization, benzofuran **16** was treated with chlorosulfonic acid at −78 °C in nitropropane. Under these conditions, cyclization was complete within 30 min yielding **18** in 43% yield as a 2/5 mixture of the C-8 epimers **18a** and **18b**.

The aldehyde functionality was introduced by treatment of the isomeric mixture of bromobenzenes **18** with *n*-butyllithium to give the corresponding phenyllithium mixture that was condensed with DMF followed by hydrolysis. The resulting aldehydes **19a** and **19b** were separated by normal-phase HPLC to give the desired product **19a**, which had the trans 6,7 ring junction and the Me-21 α configuration. Compound **19b** obtained from the HPLC purification crystallized and X-ray diffraction analysis confirmed that it had the Me-21 β configuration. Demethylation of **19a** with BI_3 followed by reductive workup with aqueous sodium thiosulfate cleanly generated racemic liphagal (**1**) that was identical to the natural product by HPLC, ^{1}H NMR, and ^{13}C NMR analyses.

Scheme 3. Synthesis of Liphagal (**1**) from Intermediate **16**



The biomimetic synthesis of liphagal (**1**) efficiently assembled the tetracyclic skeleton of the natural product with stereoselective formation of the trans fusion between the six- and seven-membered rings. It provides strong chemical support for the proposal that the biogenesis of liphagal might involve the use of a benzofuran residue to guide a biological cation-initiated polyene cyclization reaction toward the formation of the fused 6,7 ring substructure. Liphagal (**1**) is a more potent inhibitor of PI3K α than LY294002 (**3**), more selective than wortmannin (**2**), and shows significant in vitro cytotoxicity against a small panel of human tumor cell lines, making it a promising lead structure for the development of a new class of PI3K inhibitors.

Acknowledgment. Financial support was provided by a NIH-NCDDG grant (CA 67786). F.M. was supported by a Fellowship from Pierre Fabre.

Supporting Information Available: Experimental details, NMR assignments for liphagal, NMR spectra of **1** and synthetic internediates, and an ORTEP drawing for **19b**. This material is available free of charge via the Internet at http://pubs.acs.org.

OL052744T

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