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Synthesis of Pelorol and Analogues: Activators of the Inositol 5-Phosphatase SHIP

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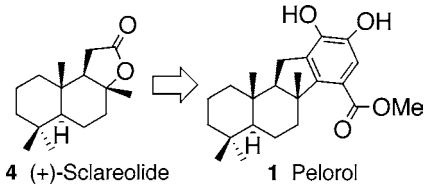
ABSTRACT



4 (+)-Sclareolide **1** Pelorol

A screening program designed to find new antiinflammatory agents has identified the sponge meroterpenoid pelorol (1) as an in vitro activator of the inositol-5-phosphatase SHIP. Pelorol (1) and several functional group analogues have been synthesized from sclareolide (4).

The phosphatidylinositol-3-kinase (PI3K) signaling pathway plays an important role in the regulation of many cellular functions, including survival, adhesion, movement, proliferation, differentiation, and end cell activation.[1] A key second messenger in this pathway is the membrane-associated phosphatidylinositol-3,4,5-trisphosphate (PI-3,4,5-P_3), which is present in low levels in unstimulated cells but is rapidly synthesized from PI-4,5-P_2 by PI3K in response to a diverse set of extracellular stimuli (Figure 1). To ensure that activation of the PI3K pathway is appropriately restrained, the tumor suppressor PTEN hydrolyzes PI-3,4,5-P_3 back to PI-4,5-P_2 and the Src homology 2-containing inositol 5-phosphatases SHIP, sSHIP, and SHIP2 hydrolyze it to PI-3,4-P_2. Approximately 50% of human cancers contain biallelic inactivating mutations of the ubiquitously expressed PTEN, which illustrates the importance of these phosphatases in preventing uncontrolled cell growth. Similar to PTEN, SHIP2 is expressed in a wide variety of cell types, whereas sSHIP is restricted to stem cells and SHIP is found only in hematopoietic (blood) cells.

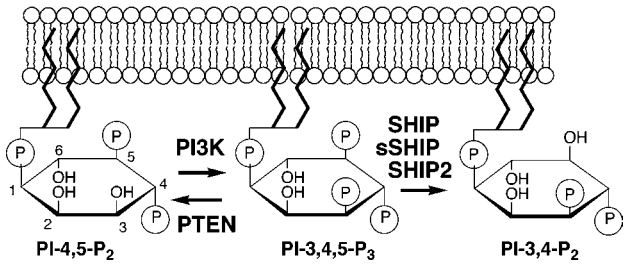


Figure 1. Enzymatic synthesis and degradation of PI-3,4,5-P_3.

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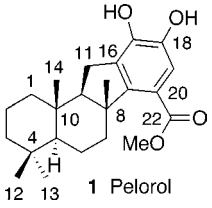
Krystal and co-workers have generated mice containing a homozygous deletion of SHIP (SHIP−/− mice).[2] These animals are viable and fertile but typically do not survive beyond 14 weeks because of a myeloproliferative disorder. Experiments with SHIP−/− mice and with SHIP−/− bone-marrow-derived mast cells (BMMCs) and macrophages (BMmϕs) obtained from these mice, have demonstrated that SHIP is a negative regulator of immunoglobulin E (IgE) or Steel Factor induced mast cell activation,[3] a negative regulator of lipopolysaccharide (LPS) induced macrophage activation, and a negative regulator of osteoclast formation and resorptive function. As a result of this last property, SHIP−/− mice suffer from severe osteoporosis.[4] There is also evidence that SHIP acts as a tumor suppressor in both acute myelogenous leukemia (AML)[5] and in chronic myelogenous leukemia (CML).[6]

Current attempts to develop drugs based on intervention in signaling pathways are overwhelmingly biased toward finding selective kinase inhibitors. There has been some recent interest in examining the therapeutic potential of phosphatase inhibitors,[7] but there has been virtually no effort to explore the usefulness of small-molecule phosphatase activators. The important role of SHIP as a negative regulator of mast cell and macrophage activation, osteoclast formation, and resorptive function, as well as in AML and CML, combined with its occurrence only in hematopoietic cells, makes it an attractive drug target. We hypothesized that selective activators of SHIP would be useful experimental tools and potential drug candidates to provide proof of principle validation for a new approach to the treatment of inflammation, osteoporosis, and leukemia.

Crude extracts of marine invertebrates were screened for in vitro activation of the SHIP-catalyzed conversion of inositol-1,3,4,5-tetrakisphosphate (IP_4) to inositol-1,3,4-trisphosphate (IP_3).[8] A MeOH extract of the sponge *Dactylospongia elegans* (Thiele, 1899), collected in Papua New Guinea,[9] showed promising activity in the assay. Bioassay-guided fractionation of the extract identified pelorol (**1**) as the sole SHIP-activating component. Three related mero-



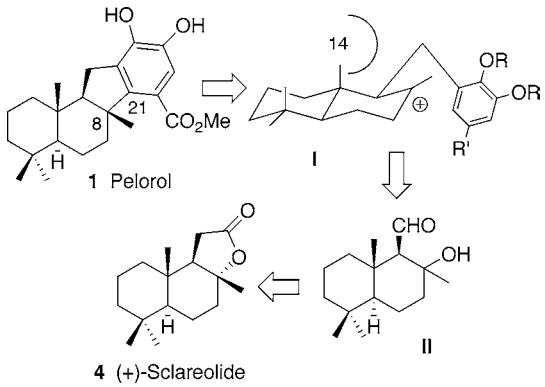
1 Pelorol

terpenoids, illimaquinone,[10] mamanuthaquinone,[11] and dactyloquinone A,[12] were also isolated from the *D. elegans* extract but were not active in the assay. Pelorol (**1**) was undescribed when first isolated in our laboratory as a SHIP activator, but while further biological studies were in progress it was isolated by Konig's group, also from *D. elegans*,[13] and by Schmitz's group from *Petrosaspongia metachromia*.[10] Spectroscopic data obtained for pelorol in the current work was in complete agreement with the data reported by Konig and Schmitz.

The limited quantity (~10 mg) of pelorol (**1**) available from the source sponge *D. elegans* was inadequate to support detailed in vitro and in vivo evaluation of its ability to activate SHIP. To satisfy the need for additional material, confirm the absolute configuration of the natural product, and generate analogues for SAR, the total synthesis of pelorol (**1**) and analogues where the methyl ester at C-20 was replaced by methyl and ethyl residues was undertaken.

On the basis of sound biogenetic arguments, Schmitz predicted that the absolute configuration of pelorol (**1**) was 5*S*,8*R*,9*R*,10*S* as drawn. Therefore, the starting material selected for the synthesis of pelorol and analogues was the commercially available terpenoid (+)-sclareolide (**4**), which has the same absolute configurations at C-5, C-9, and C-10 as those predicted for pelorol (**1**). The synthetic plan anticipated that the key reaction would involve a biomimetic carbocation-initiated cyclization of an intermediate **I** to generate the C-8/C-21 bond (Scheme 1). Steric bulk associ-

Scheme 1. Retrosyntheic Analysis of Pelorol (**1**)



ated with the C-14 methyl was expected to cause preferential approach of the phenyl ring from the bottom face of C-8 to form the required trans B/C ring fusion. Synthetic routes to both C-8 epimers (**5** and **15**) of **II** starting from sclareolide have been reported, and the plan was to examine both as

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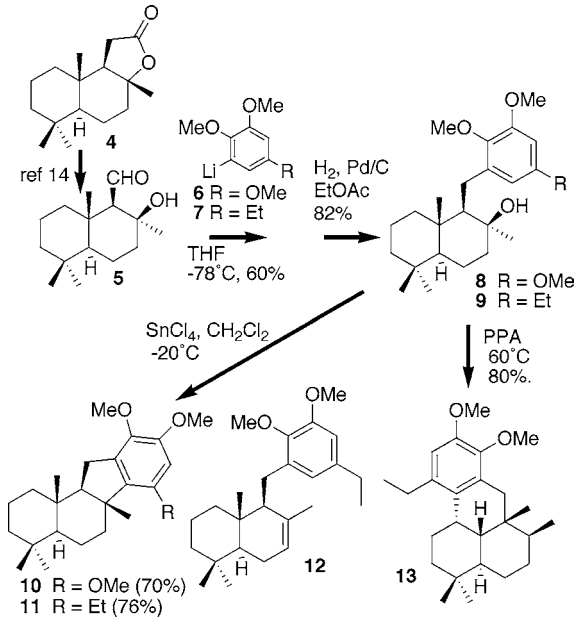
(8) The SHIP assay was performed in 96-well microtitre plates with 10 ng of recombinant SHIP enzyme per well. SHIP enzyme was incubated with extract or DMSO for 15 min at 23 °C before addition of 200 mM inositol-1,3,4,5-tetrakisphosphate. The reaction was allowed to proceed for 20 min at 37°C and the amount of inorganic phosphate released was then assessed by the addition of Malachite Green reagent followed by an absorbance measurement at 650 nm.

intermediates in the preparation of direct precursors to the carbocation **I** and ultimately cyclized products.

(+)-Sclareolide (**4**) was converted to the aldehyde **5** following the literature procedure (Scheme 2).[14] Overman

Scheme 2. Initial Attempts at Biomimetic Cyclizations

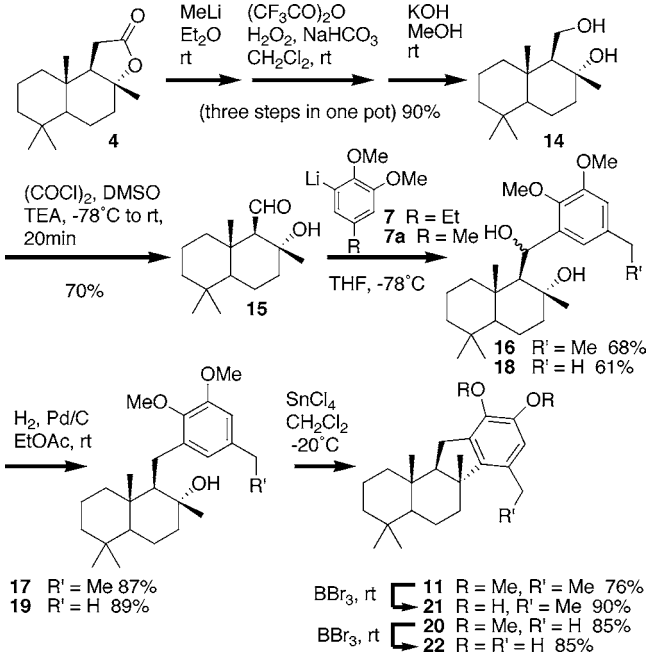


showed in his synthesis of adociasulfate that a highly nucleophilic arene was required to trap a carbocation such as **I** in preference to proton elimination to give an uncyclized olefin.[15] Therefore, trimethoxyphenyllithium **6** (2 equiv) was added to aldehyde **5**, followed by hydrogenolysis of the resulting epimeric benzyl alcohols, to give the tertiary alcohol **8**. Treatment of **8** with $SnCl_4$ gave the cyclization product **10** having the desired stereochemistry in good yield. Unfortunately, all attempts to transform 1,2,4-trialkoxy benzene **10** or various alkyl ether analogues into pelorol failed.

In an attempt to overcome this problem, the coupling reaction was repeated with dimethoxyethylphenyllithium **7** to give the tertiary alcohol **9**. Initial reactions of **9** with $SnCl_4$ gave variable yields of the desired product **11** and the undesired elimination product **12**, while treatment with the protic acid PPA gave only the unanticipated cyclization product **13**. We envisaged that the formation of **12** and **13** resulted from reduction of the nucleophilicity of the arene (R = Et vs R = OMe) making the elimination reaction leading to **12** and the Wagner Meerwein rearrangements leading to **13** competitive with direct trapping of the C-8 carbocation by the arene to give the desired product **11**. After optimization of the reaction conditions, it was found that the $SnCl_4$-catalyzed cyclization gave consistently high yields (~76%) for the conversion of **9** to **11**.

Although it was possible to obtain **11** via the aldehyde **5**, the preparation of **5** from sclareolide was cumbersome because it required multiple chromatographic separations and preparation of an oxidizing agent that was not commercially available.[14] Therefore, we turned our attention to the aldehyde **15**, having an equatorial OH group at C-8 (Scheme 3).[16] (+)-

Scheme 3. Synthesis of the Cyclized Intermediate **11**



Sclareolide (**4**) was converted to the diol **14** in excellent yield (90%) using a one-pot three-step sequence modification of the literature procedure.[16] Swern oxidation cleanly oxidized the diol **14** to the aldehyde **15**.[17] Reaction of **15** with phenyllithium **7** gave the epimeric benzyl alcohols **16** in good yield. Hydrogenolysis of the mixture **16** cleanly removed the benzylic alcohols to give **17**. Cyclization of **17** using $SnCl_4$ as a catalyst gave the desired tetracyclic intermediate **11** in high yield, without any trace of the elimination product **12**. Dimethyl ether **11** was converted to the catechol **21**, and the entire sequence was repeated with phenyllithium **7a** to give the methyl analogue **22** to provide pelorol analogues for SAR.

Reaction of **11** with PCC selectively oxidized the C-22 methylene to give methyl ketone **23** in reasonable yield (Scheme 4). Treatment of **23** with I_2 in aqueous NaOH, in

(9) A voucher sample has been deposited at the Zoological Museum, University of Amsterdam (ZMA POR. 15986).

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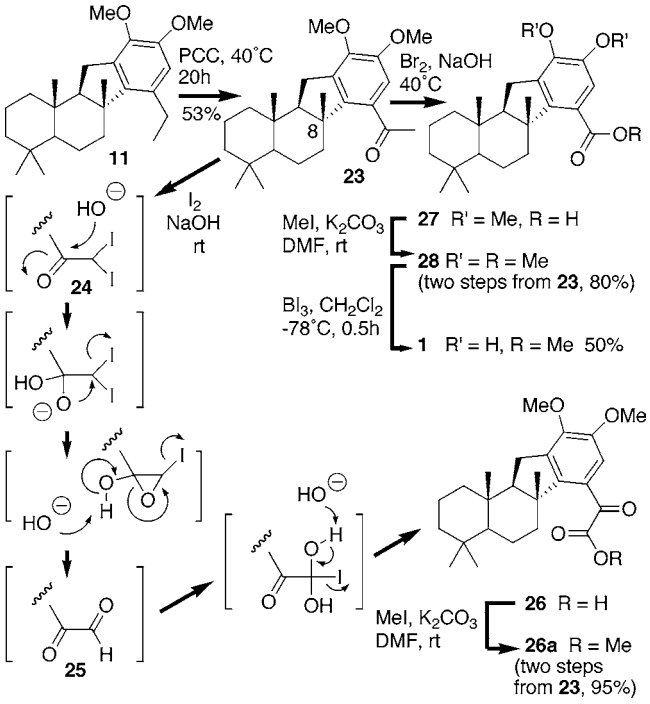
Scheme 4. Synthesis of Pelorol (**1**)



an attempt to effect a haloform reaction to give benzoic acid **27**, unexpectedly resulted in the near quantitative formation of the α-ketoacid **26**. This anomalous result might be caused by the steric bulk of the C-8 carbon ortho to the C-22 ketone preventing the formation of a triiodinated methyl. If a diiodinated methyl ketone **24** is attacked by hydroxide to give a tetrahedral intermediate, the diiodomethyl may not be a good enough leaving group to depart in the normal fashion to give a carboxylic acid. Instead, an intramolecular S_N2 displacement of iodide can form an epoxide, which after fragmentation as shown in Scheme 4 can lead to the α-keto aldehyde **25**. Oxidation of **25** via iodination of the aldehyde hydrate can generate the final α-ketoacid **26**. Simply changing the halogen to Br_2 led to a clean transformation of methyl ketone **23** to the desired benzoic acid **27**.

The synthesis of pelorol (**1**) was completed by esterification of **27** with MeI followed by selective cleavage of the phenyl methyl ethers with BI_3 at −78 °C. Synthetic pelorol (**1**) was identical by NMR and MS comparison with the natural product. The $[\alpha]_D$ of the synthetic material was −64° compared with values of −69° reported by Konig and −71° reported by Schmitz, confirming that the absolute configuration is 5*S*,8*R*,9*R*,10*S* as predicted by Schmitz.[10]

Pelorol (**1**), dimethylpelorol (**28**), the analogues **21** and **22**, the corresponding methyl ethers **11** and **20**, the trimethoxy pelorol analogue **10**, and the uncyclized precursor **19** were tested for in vitro activation of SHIP and the ability to suppress degranulation and tumor necrosis factor α (TNFα) production in murine mast cells stimulated with IgE.[18] Synthetic pelorol (**1**), the ethyl analogue **21**, and the methyl analogue **22** showed significant activity in all three assays, but the methyl ethers **10**, **11**, **19**, **20**, and **28** were inactive. The relative effectiveness of the active compounds in the SHIP activation assay was **22** > **21** ≈ **1**, showing that replacement of the methyl ester at C-20 in pelorol with a methyl gives enhanced activity. Lack of activity in the dimethyl ethers **20**, **11**, and **28** demonstrates that at least one phenol is required for activity.

Compound **22** was chosen for further evaluation because it showed biological activity greater than that of pelorol and its synthesis was shorter. Side by side evaluation of **22**'s ability to suppress degranulation and TNFα release in SHIP−/− and SHIP+/+ mast cells showed that it was only active in the SHIP+/+ cells, indicating that it selectively targets SHIP. Compound **22** also showed positive effects comparable to the reference standard dexamethasone in a standard mouse ear edema assay for topical antiinflammatory activity and in a mouse model of septic shock.[18]

In summary, the sponge meroterpenoid pelorol (**1**) has been identified as an activator of the inositol-5-phosphatase SHIP. An efficient synthesis of pelorol (**1**) and several analogues has confirmed the structure and absolute configuration of the natural product and provided preliminary SAR for the SHIP-activating pharmacophore. The C-20 methyl analogue **22** has shown promising in vivo activity in two mouse models of inflammation, supporting the initial hypothesis that selective small-molecule SHIP activators should represent a new class of antiinflammatory agents.

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

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(18) Pure compounds were tested at 5 μg/mL. In the SHIP assay, **22** showed >6-fold activation, whereas **1** and **21** showed ca. 2-fold activation. See Supporting Information for experimental details of the in vivo assays. A complete description of the biological activity of pelorol and the synthetic analogues will be published elsewhere.