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Total Synthesis of Asperphenins A and B

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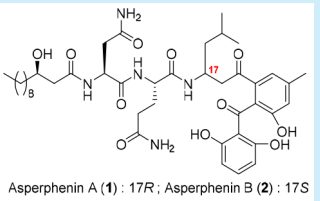
Supporting Information

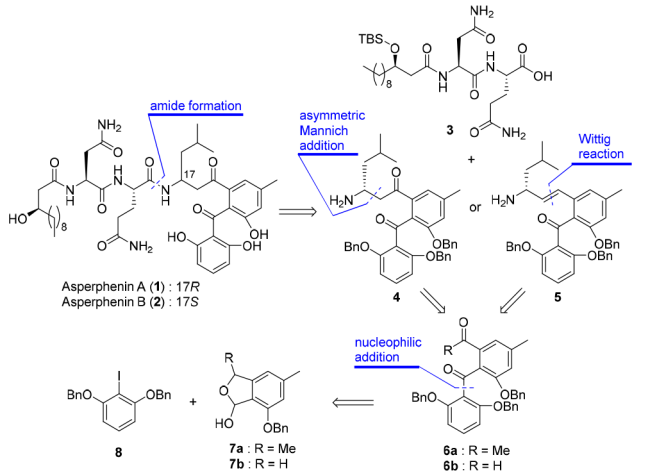
ABSTRACT: The first total synthesis of asperphenins A and B has been accomplished in a concise, highly stereoselective fashion from commercially available materials (15 steps, 9.7% and 14.2% overall yields, respectively). The convergent route featured the judicious choice of protecting groups, fragment assembly strategy and a late-stage iron-catalyzed Wacker-type selective oxidation of an internal alkene to the corresponding ketone.



Metabolites isolated from marine fungus often possess unique structural features and incorporate new or unusual assemblages of functional groups. Many of them provide novel lead molecules for probing fundamental biological processes and the development of novel chemotherapeutic agents that target cancers. Our laboratory is engaged in a program devoted to the total synthesis and evaluation of marine natural products.[1] Herein, we disclose the first total synthesis of asperphenins A and B by utilizing a highly efficient and convergent approach.

Asperphenins A and B (Scheme 1) were isolated from a culture broth of marine-derived *Aspergillus* sp. collected from the shore of Jeju Island, Korea.[2] The relative and absolute stereochemical configuration of asperphenins had been established by a combination of spectroscopic analyses, chemical degradation, Mosher ester analysis, CD measurements and ECD calculations. Structurally, these two natural products are composed of a β-hydroxy fatty acid, a tripeptide, and a trihydroxybenzophenone. Asperphenins A and B exhibit significant cytotoxicity against several human cancer cell lines, e.g., with IC_{50} values of 0.8 and 1.1 μM, respectively, against RKO colorectal carcinoma cells.

Scheme 1. Retrosynthetic Analysis of Asperphenin A



Scheme 1 outlines our retrosynthetic analysis plan. We anticipated that the condensation of acid **3** with either fragment **4** or **5** followed by removal of protecting groups would give rise to the natural products. We envisioned the amine moiety of **4** or **5** could arise from either asymmetric Mannich reaction of ketone **6a** or a stereoselective Wittig olefination of aldehyde **6b** with a leucinol-derived triphenylphosphonium salt. Both **6a** and **6b**, in turn, could be accessed from a reaction of aryl iodide **8** with lactols **7a** and **7b**, respectively.

The synthesis of fragment **3** commenced with the known compound **9**,[3] which underwent titanium tetrachloride mediated enantioselective aldol reaction with caprinaldehyde to provide **10**[4] as a single diastereomer in 81% yield (Scheme 2). Protection of the resulting alcohol as its TBS ether followed by hydrolytic cleavage of the chiral auxiliary furnished the acid **11** in 76% yield. In parallel, coupling of *N*-Cbz-L-asparagine and L-glutamine methyl ester under the influence of HATU in the presence of HOAt and DIPEA furnished the corresponding dipeptide, which was subjected to hydrogenolysis of the Cbz protecting group to produce amine **14** in 76% yield over two steps. A second HATU/HOAt-mediated condensation of acid **11** and amine **14** provided the corresponding amide **15** in 85% yield. Saponification of the methyl ester with LiOH in aqueous methanol followed by acidification afforded the acid **3** in 81% yield.

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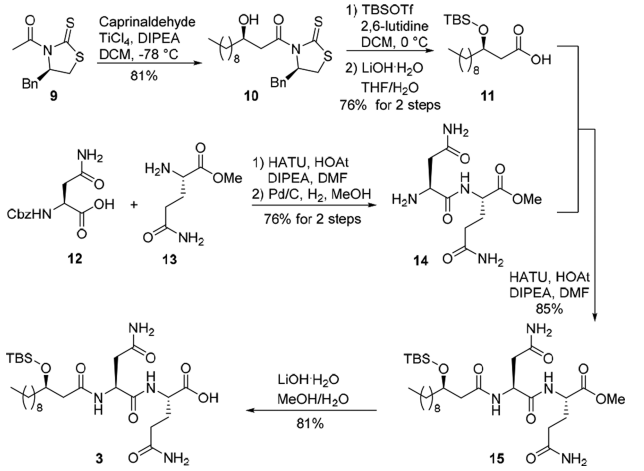
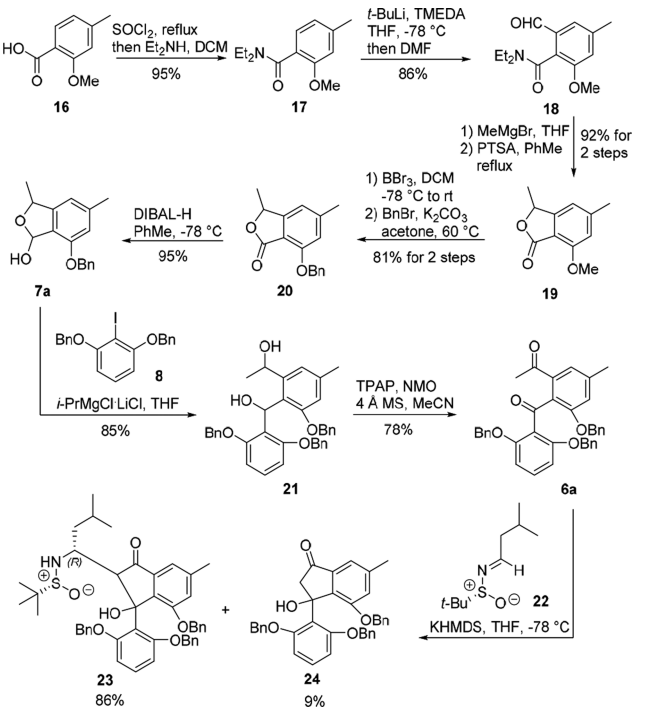
Scheme 2. Synthesis of Fragment 3



Our planned first approach to asperphenin A required the synthesis of fragment **4** from ketone **6a** via an asymmetric Mannich reaction (Scheme 3). Thus, 2-methoxy-4-methyl-

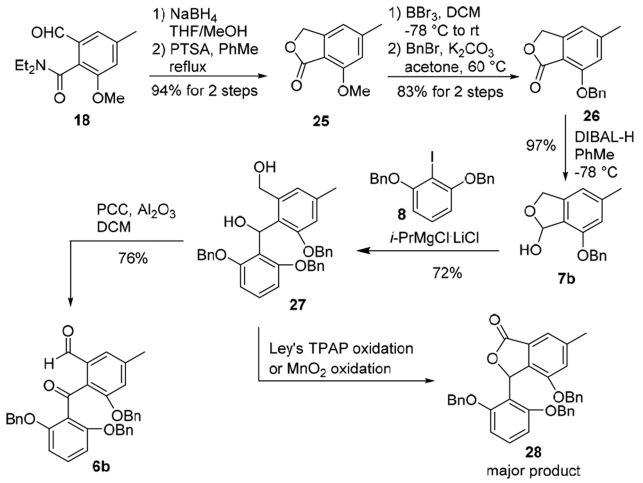
Scheme 3. Attempts on the Synthesis of Intermediate 4



benzoic acid **16** was converted into the corresponding acid chloride and then coupled with *N*,*N*-diethylamine to afford **17** in 95% yield. Regioselective formylation of **17** to aldehyde **18** was achieved through an amide-directed *ortho*-lithiation (*t*-BuLi, TMEDA) and quenching with DMF (86% yield).[5] Addition of methylmagnesium bromide to aldehyde **18** afforded the corresponding secondary alcohol, which was immediately subjected to acid-promoted lactonization to give rise to **19** in 92% yield over two steps.[5b,6] Lactone **19** was treated with boron tribromide in dichloromethane to produce the crude deprotected phenol, which was then reprotected by benzylation using benzyl bromide and potassium carbonate to afford the benzyl derivative **20** in 81% yield. Partial reduction of the lactone **19** using DIBAL-H at −78 °C furnished the corresponding lactol **7a** as a latent hydroxyaldehyde in 95% yield. Spurred by Knochel's seminal findings on halogen−magnesium exchange,[7] we opted to convert the readily available aryl iodide **8** to the corresponding arylmetal species with *i*-PrMgCl·LiCl, followed by the addition of lactol **7a** to give rise to biphenyl diol **21** as a mixture of diastereomers in 85% yield. Ley's TPAP oxidation[8] of **21** delivered the corresponding diketone **6a** in 78% yield and set the stage for the exploration of the key asymmetric Mannich reaction.[9] However, this reaction proved to be challenging due to the high propensity of trapping of the transient enolate intramolecularly with an electrophile. Accordingly, treatment of methyl ketone **6a** and *tert*-butanesulfinyl imine **22** with various bases, solvents, and concentrations at low temperature resulted in no observation of the desired product. For example, exposure of both **6a** and **22** in the presence of 1 equiv of potassium hexamethyldisilazane (KHMDS) provided two aldol products (**23** and **24**). Given that the reactions afforded *β*-amino ketone **23** as the major product, we reasoned that the asymmetric Mannich reaction occurred with simultaneous cyclization of the regenerated enolate onto the ketone to afford the thermodynamically more stable product.

To circumvent the problems encountered in the above strategy toward the construction of chiral amine **4** via an asymmetric Mannich reaction, we turned our attention to the strategy where the ketone moiety would have to be installed at a later stage of the synthesis. Thus, aldehyde **18** was transformed into lactone **25** by a two-step sequence involving (1) reduction with sodium borohydride and (2) acid-promoted lactonization. Lactone **25** was then elaborated to the lactol **7b** in 81% yield by an identical strategy as described for **7a**, including methyl ether deprotection and reprotection of the resulting free phenol with benzyl bromide and potassium carbonate, followed by partial reduction of the lactone with DIBAL-H at −78 °C. Again, the Grignard reagent derived from aryl iodide **8** added to lactol **7b** provided biphenyl diol **27** in 72% yield. To our surprise, the use of the previously employed Ley's TPAP oxidation of diol **27** led to the dicarbonyl product **6b** in 27% yield along with the over-oxidation product **28** (58%) (Scheme 4). Similar results were obtained with manganese dioxide[11] in dichloromethane. 2,3-Dichloro-5,6-dicyano-1,4-benzoquinone (DDQ)[12] oxidation of the benzylic alcohols in **27** provided intractable mixtures with

Scheme 4. Synthesis of Aldehyde 6b



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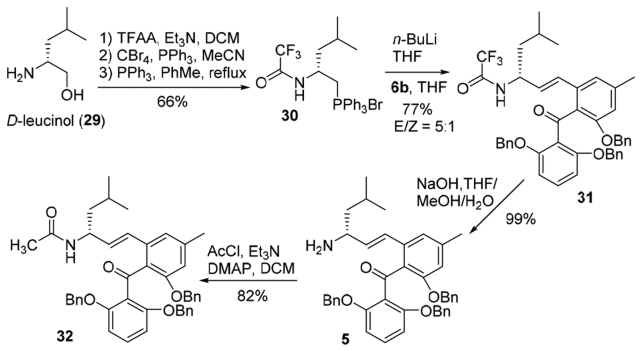
no desired products. Gratifyingly, treatment of diol **27** with PCC on alumina[13] in DCM smoothly afforded the desired aldehyde **6b** in 76% yield as the sole product.

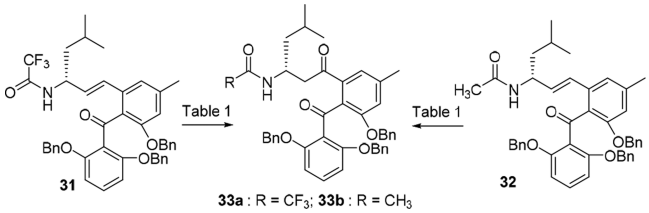
In parallel, D-leucinol (**29**) was transformed into trifluoroacetamide **30** by a three-step sequence involving (1) trifluoroacetylation of the primary amine, (2) conversion of the alcohol into the bromide with CBr_4 and Ph_3P, and (3) β-amino phosphonium salt formation upon treating the resulting bromide with triphenylphosphine in refluxing toluene. Wittig reaction of aldehyde **6b** with the phosphorane derived from phosphonium bromide **30** was next examined. Thus, treatment of the phosphonium salt **30** with 2.1 equiv of *n*-butyllithium followed by addition of the aldehyde **6b** delivered the alkene **31** enriched in the desired *E*-isomer (E/Z = 5:1).[14] The desired product **31** could be isolated free of the Z isomer in 77% yield by chromatography on silica gel. The presence of the trifluoroacetamide in **30** ensured the generation of the dianion intermediate, which presumably played a critical role to the stereochemical outcome of this transformation.[14b,15] Hydrolysis of the trifluoroacetamide **31** gave allylic amine **5** in nearly quantitative yield. Acetylation of amine **5** was achieved under basic conditions with acetyl chloride to afford **32** in 82% yield (Scheme 5).

Scheme 5. Synthesis of Amine 5 and Amides 31 and 32



With alkenes **31** and **32** in hand, the stage was set to explore the crucial Wacker-type oxidation. Attempts to selectively oxidize the *E*-alkenes **31** and **32** using molecular iodine or *N*-iodosuccinimide[16] gave little or no desired products (Table 1, entries 1−4). The literature precedents suggested that Wacker oxidation of cinnamyl azides[17a] and 2-styryltetrahydro-2*H*-pyrans[17b] occurred predominantly or exclusively at the benzylic rather than the homobenzylic carbon. Attempted Wacker oxidation of both **31** and **32**, however, delivered only trace quantities of the desired ketone. As we searched for alternative methods of transforming alkenes **31** and **32** into ketones **33a** and **33b**, we were drawn to a report by Han and co-workers of a mild variant of the Wacker-type oxidation.[18] In the Han's modification of the Wacker oxidation, iron(II) chloride, polymethylhydrosiloxane, and air were employed as a highly efficient and selective catalytic system. Because the mild oxidation conditions enable exceptional functional-group tolerance, we tested this protocol with alkene **31**. We were pleased to discover that the iron-catalyzed Wacker-type oxidation of alkene **31** provided ketone **33a** in 30% yield. To our delight, the same iron-catalyzed aerobic oxidation of **32** delivered **33b** in 89% yield (Table 1, entries 7 and 8).

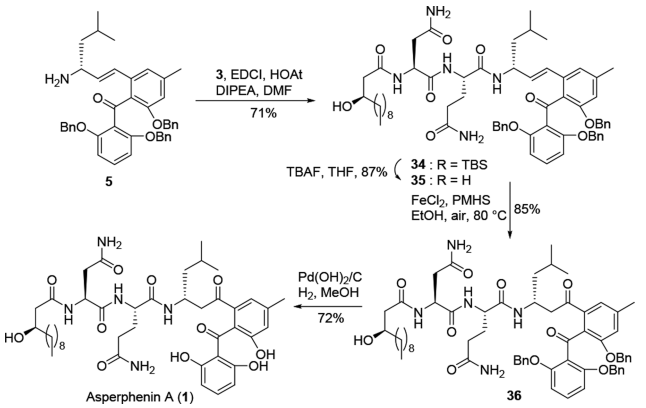
Table 1. Optimization of the Wacker-Type Oxidation



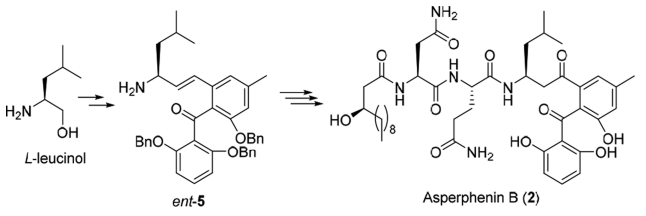
entry	alkene	condition	temp (°C)	yield (%)
1	**31**	I_2, dioxane/H_2O	90	ND
2	**32**	I_2, dioxane/H_2O	90	ND
3	**31**	NIS, dioxane/H_2O	30	trace
4	**32**	NIS, dioxane/H_2O	30	trace
5	**31**	$PdCl_2$, CuCl, O_2, H_2O, DMF	50	trace
6	**32**	$PdCl_2$, CuCl, O_2, H_2O, DMF	50	trace
7	**31**	$FeCl_2$, PMHS, EtOH, air	80	30
8	**32**	$FeCl_2$, PMHS, EtOH, air	80	89

Encouraged by the successful transformation of alkene **32** to the corresponding β-ketone amide **33b**, we proceeded with the total synthesis of asperphenin A and B as outlined in Schemes 6 and 7. Thus, coupling of acid **3** and allylic amine **5** under the influence of EDCI in the presence of HOAt and DIPEA afforded **34** in 71% yield. After removal of the TBS group to give rise to the corresponding alcohol **35**, the alcohol was then subjected to an iron-catalyzed Wacker oxidation to furnish **36** in 85% yield. The final global hydrogenative debenzylation went on without incident to provide asperphenin A in 72% yield (Scheme 6). To this end, we synthesized *ent*-**5** following the same synthesis as for **5**, but starting with L-leucinol. This was readily achieved, and *ent*-**5** was incorporated into the synthesis as previously performed to afford asperphenin B with no adverse consequences (Scheme 7). The spectral data for synthetic **1** and **2** (^1H and ^{13}C NMR and HMRS) were identical with those published for the natural products, and the optical rotation of our products ($[\alpha]_D^{25}$ −22.0, *c* 0.1, MeOH,

Scheme 6. Synthesis of Asperphenin A



Scheme 7. Synthesis of Asperphenin B



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for asperphenin A; $[\alpha]_D^{25}$ −16.2, *c* 0.1, MeOH, for asperphenin B) corresponded well with the literature value (lit.[2] $[\alpha]_D^{25}$ −24.7, *c* 0.1, MeOH, for asperphenin A; $[\alpha]_D^{25}$ −18.4, *c* 0.1, MeOH, for asperphenin B), leading us to conclude that synthetic **1** and **2** were of the same absolute stereochemistry as natural asperphenins A and B.

With the synthetic asperphenins A (**1**) and B (**2**) in hand, the screening of cytotoxic activities toward a number of cancer cell lines was investigated. The initial cytotoxicity evaluation of **1** and **2** was performed across a panel of the HIF-dependent HCT116 colorectal cancer cell lines using isogenic ($HCT116^{HIF\text{-}1\alpha-/-HIF\text{-}2\alpha-/-}$ and $HCT116^{WT\ KRAS}$) knock-out cells to identify if these compounds preferentially target HIF/KRAS pathways (Figure 1). Both compounds were only

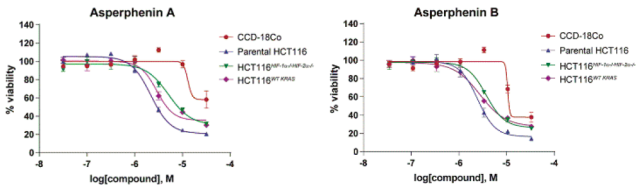

Figure 1. Effect of asperphenins A (**1**) and B (**2**) on isogenic HCT116 colon cancer cells and normal colon cells.

moderately cytotoxic against parental HCT116 with reduced cytotoxicity against the human normal colon cells, CCD-18Co.[10] Asperphenin A (**1**) shows a 2-fold decrease in potency against $HCT116^{HIF\text{-}1\alpha-/-HIF2\alpha-/-}$ (IC_{50} shifts from 2.2 uM to 5.1 μM) with a simultaneous decrease in efficacy. For cells lacking oncogenic KRAS ($HCT116^{WT\ KRAS}$), only a slight decrease in total efficacy was observed compared to the parental HCT116 cell line.[10] Asperphenins may have a slight selectivity for colon cancer cells over normal cells that could be explored further with SAR studies.

In summary, stereocontrolled total synthesis of asperphenins A and B has been accomplished through combination of a judicious choice of protecting groups, a fragment assembly strategy, and a late-stage iron-catalyzed Wacker-type selective oxidation of an internal alkene to the corresponding ketone. Both of the synthetic samples exhibit interesting results in the preliminary biological assays.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.8b02652.

Experimental details and data (PDF)

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Notes

The authors declare no competing financial interest.

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