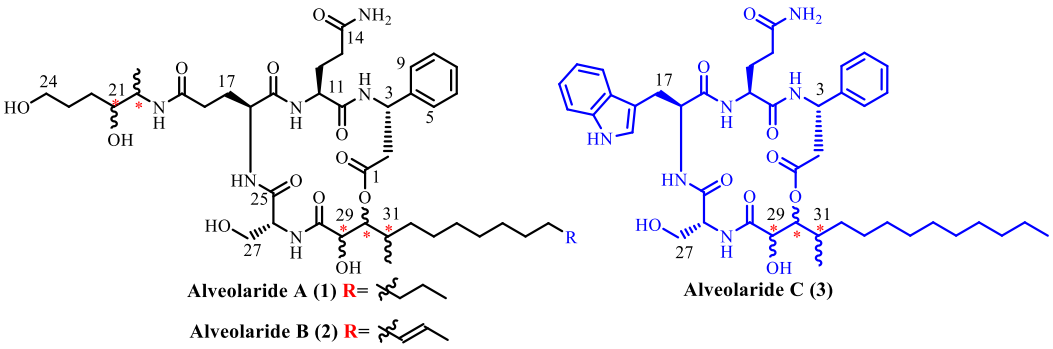
Details of the research work duly signed by the applicant, for which the Sun Pharma Science Foundation Research Award is claimed, including references and illustrations (not to exceed 6000 words).

Natural products are broadly defined as chemicals produced by living organisms, have always been at the center of attention in the scientific community, and interest in them is constantly growing. Natural products have evolved over millions of years and acquired a unique chemical diversity, which consequently results in the diversity of their biological activities and drug-like properties. Therefore, even before the rise of the modern chemical pharmacology, natural products have been used for centuries as components of traditional medicines. In modern pharmacology, natural products have become one of the most important resources for developing new lead compounds and scaffolds. Major classes of antibiotics and antifungals are based on natural products isolated from microorganisms. Drugs used in the treatment of various cancers, cardiovascular diseases, diabetes, and more are often natural products or their derivatives. Moreover, the term "*natural product"* has also been extended for commercial purposes to refer to cosmetics, dietary supplements, and foods produced from natural sources without added artificial ingredients. Our group has put considerable footmark on the field of general organic synthesis, particularly in the asymmetric total synthesis of bioactive natural products. Our research covers a wide spectrum of chemical synthesis of natural products, leading to the total synthesis of various natural products, many of which have been evaluated for biological assessment. Among the numerous numbers of synthesized natural products, the development of synthetic routes for **alveolaride C**, **beauveamide A**, **pestalotioprolides E** & **F**, and **bipolamide A** acetate is potentially valuable from the point of view of basic organic and applied chemistry. The routes developed for the aforementioned bioactive natural products are flexible, and a variety of non-natural analogues may be diversified from that to investigate their biological importance.

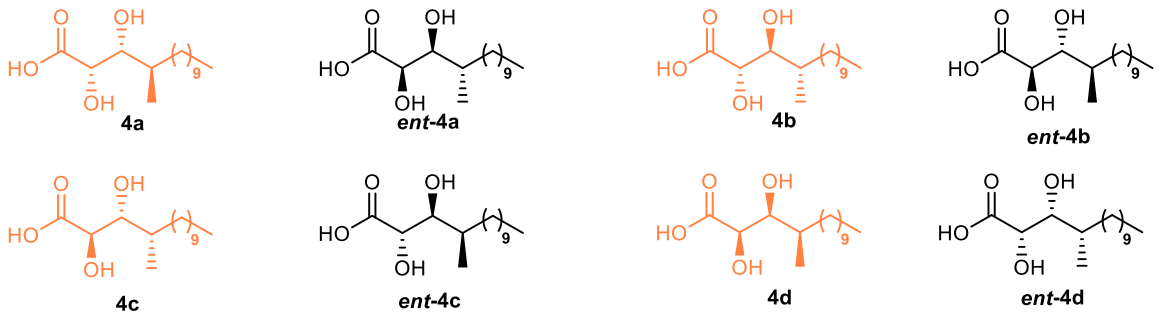
(i) In search for environment friendly pesticides present in naturally occurring microorganisms, structurally novel cyclodepsipeptides alveolarides A–C (1-3, Figure 1) were first discovered and isolated from the cultures of *Microascus alveolaris* strain PF1466 in 2018 by the researcher of Dow Agro Sciences (DAS), an agency from USA. These cyclodepsipeptides demonstrated excellent to moderate efficacies in *vitro* inhibitory experiments against a variety of hazardous plant infections, including *Pyricularia oryzae*, *Zymoseptoria tritici*, and *Ustilago maydis*. The

Figure 1. *Putative Structures of Alveolarides A-C (**1-3**).*



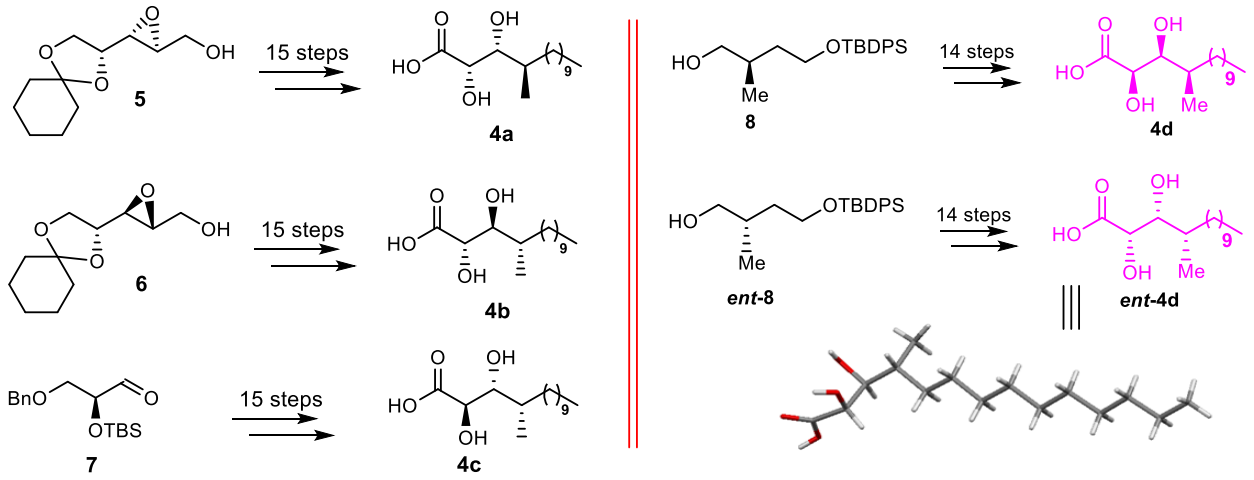
structures of Alveolarides A-C (Figure 1) could not be fully resolved by the DAS group. The partial structure of these compounds was discovered by chemical modification, mass spectroscopic analysis and NMR. The chemical structure of alveolaride C was predicted based on a comparison of ^{1}H and ^{13}C NMR data reported for alveolaride C and two other members of the family. Due to the scarcity of compound, it was not possible to carry out other degradation studies for alveolaride C. They were found to be 17-membered macrocyclic compounds with a rare non-peptide unit, 2,3-dihydroxy-4-methyltetradecanoic acid (DHMTDA). The stereochemistry of the asymmetric centers of the DHMTDA segment are still unknown. We targeted alveolaride C (Figure 1) with an intension to solve its structural mystery using total synthesis as a state of art. DHMTDA (**7**) unit contains three unknown stereocenters and it was conceived to match with one out of eight possible stereoisomers [**4**(**a**-**d**) and ***ent*-4**(**a**-**d**), Figure 2] and from this finding the actual structure of alveolaride C was quite challenging. We initially planned to synthesize four of them **4**(**a**-**d**) with the intention of comparing their spectroscopic data with the reported data of the DHMTDA segment to solve the structural puzzle.

Figure 2. *Possible Stereoisomers of DHMTDA [**4(a–d)** and **ent-4(a–d)**].*



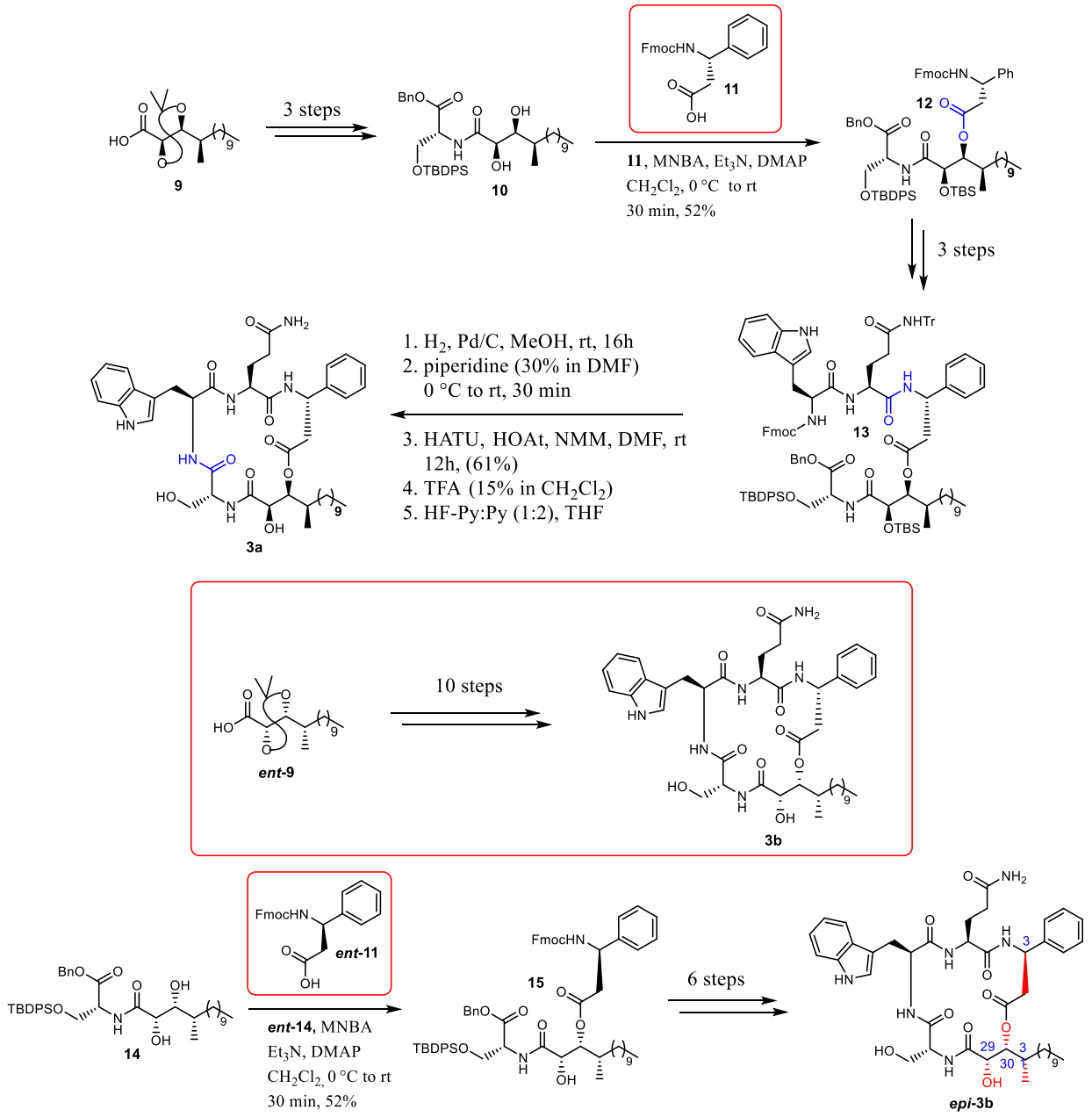
Accordingly, four acids **4**(**a–d**) (Figure 2) were synthesized (Scheme 1) using Sharpless asymmetric epoxidation and asymmetric dihydroxylation, Julia–Kocienski olefination, Crimmins aldol reactions, Pinnick oxidations, and manipulations of protection–deprotection chemistry as primary synthetic weapons. Comparison of ^{1}H and ^{13}C NMR suggest that acid **4d** was the best match among the synthesized isomers. However, since the optical rotation of the DHMTDA unit was not reported by the isolating group, the enantiomer ***ent*-4d** was also synthesized.

Scheme 1. *Synthesis of different diastereomeric DHMTDA [**4(a–d)** and **ent-4(a–d)**].*



We first intended to synthesize both the macrocycles carrying acid **4d** and ***ent*-4d** as DHMTDA units in order to compare their NMR data with that of the isolated alveolaride C. The synthesis of **3a** (Scheme 2) involved the coupling of acid **9** with the known D-serine counterpart followed by manipulation of protecting group to afford diol **10**. Various conditions were examined for the selective esterification of diol **10** with Fmoc-protected D-β-phenylalanine (**11**) and Shiina's protocol was found to give C-3 esterified product **12** in moderate yield (52%). Formation of ester **12** was confirmed by detailed 2D NMR analysis. The cyclic precursor **13** was then synthesized using amide chemistry, and the crucial macrocyclization was performed using HATU/HOAt/NMM/DMF. Finally, global deprotection was optimized thoroughly and it was found HF-Py:Py (1:2) and TFA/CH_2Cl_2 deprotected only the silyl ether and the trityl group, respectively. Thus, a two-step protocol was adopted for global deprotection in which the trityl and silyl groups were sequentially removed to afford compound **3a** in good overall yield. During the synthesis of compound **3b**, changes were made in the protection group strategy to avoid the

Scheme 2. *Total Synthesis of Two Purported Structures of Alveolaride C (**3a** and **3b**).*



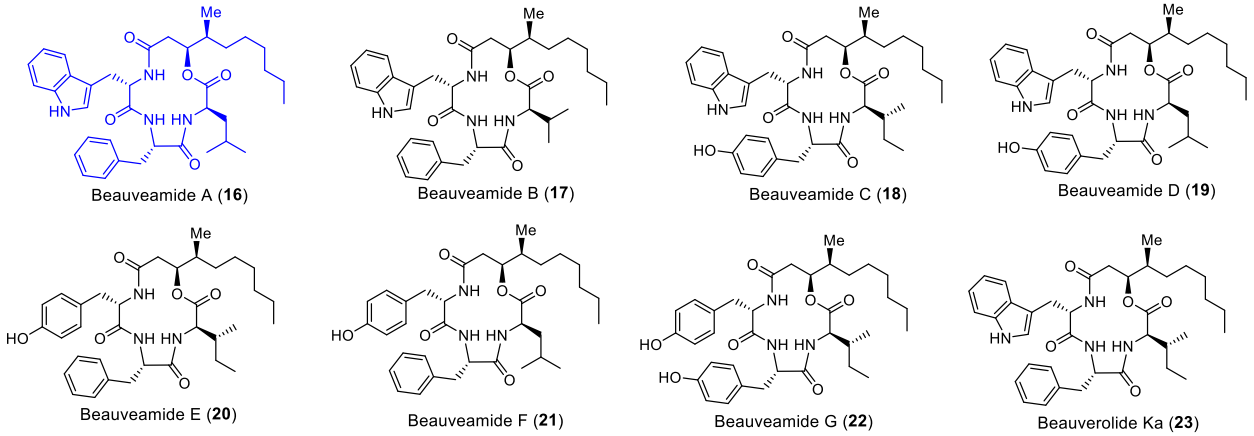
complexity in global deprotection. Compound **3b** has been prepared starting from acid ***ent*-9** (Scheme 2). The ^{1}H and ^{13}C NMR data of compounds **3a** and **3b** were compared with the data of isolated natural product. Major inconsistencies were observed for compound **3a**, while compound **3b** appeared to close with few discrepancies. Differences were observed in the ^{1}H NMR data for the protons in *β*-phenylalanine and DHMTDA counterparts. Since the structure of DHMTDA segment was confirmed by XRD study, another epimer of compound **3b** (***epi*-3b**), having the

modification in β-phenylalanine counterpart was synthesized (Scheme 2). The ^{1}H and ^{13}C NMR data of the synthesized compound ***epi*-3b** found consistent with those of the isolated natural product which confirmed the actual structure of alveolaride C. The undetermined stereocenters (C-29, C-30, and C-31) of alveolaride C successfully established and the proposed configuration of the *β*-phenylalanine (C-3) counterpart of the molecule has also been revised from *S* to *R*. This synthetic study is crucial to determine the unassigned stereocenters common to the all members of this family and also a very important stepping-stone for the total synthesis of other members of this family and their biological exploration.

(**Chem. Sci**., **2020**, *11*, 11259-11265).

(ii) Beauveamides (Figure 3) are a family of natural products with potential pharmaceutical and agrochemical value. In 2021, Puno and co-workers discovered seven new cyclotetradepsipeptides, beauveamides A-G (**16-22**) along with known beauverolide Ka (**23**) (Figure 3). Structurally these are 13-membered macrocycles having a fatty acid moiety, 3-hydroxy-4-methyldecanoic acid

Figure 3. *Structures of the Members of Beauveamide Family (**16-23**).*



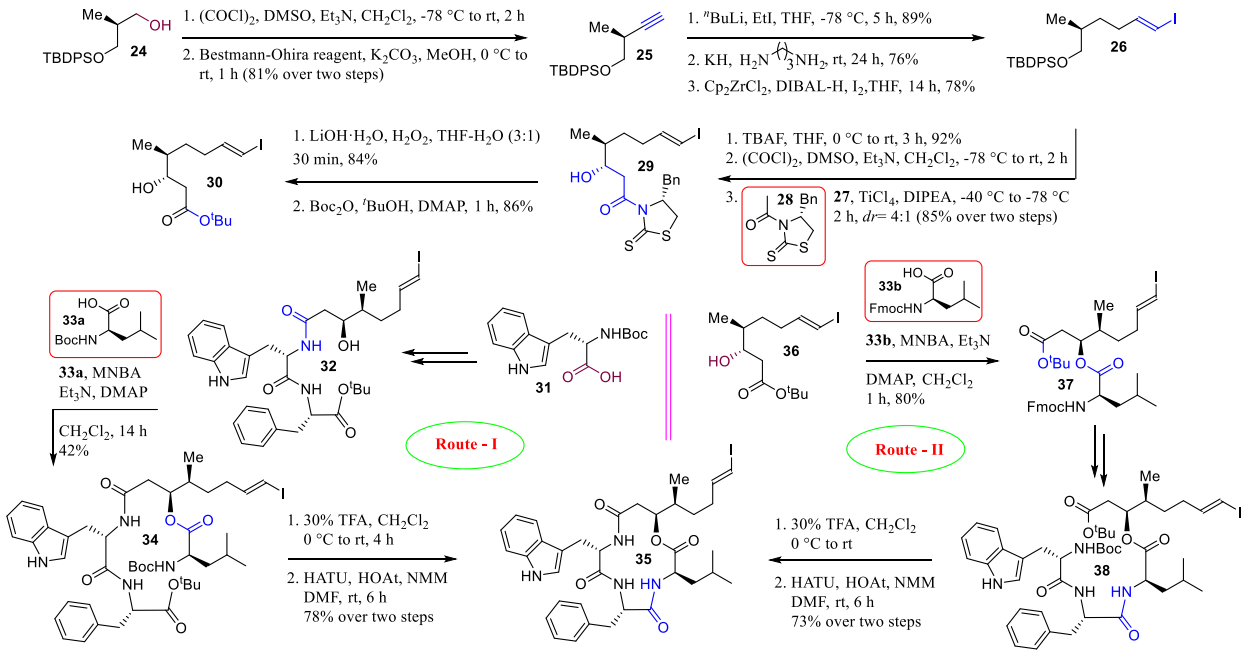
(HMDA), and three amino acids of a peptide segment which vary between members. Beauveamides A (**16**) and B (**17**) showed protective effects on the mouse auditory cell line (HEI-OC1), while beauveamides A (**16**), D (**19**), and E (**20**) stimulated glucose uptake in cultured rat L6 myoblasts. The first total synthesis of beauveamide A (**16**) was performed using a late-stage functionalization approach where a common intermediate was designed to provide various analogs with modified HMDA units for further biological studies.

Unfortunately, our initial effort involving macrolactonization approach was found

ineffective. An alternative approach for the synthesis of key intermediate **35** were adopted which relied on a macrolactamization approach. In search for the shortest synthetic path, two possible intermediates **34** (route I) and **38** (route II) have been planned which could be accessed from acid **36** and the dipeptide of L-tryptophan-L-phenylalanine and D-leucine using amidation and esterification chemistry, respectively.

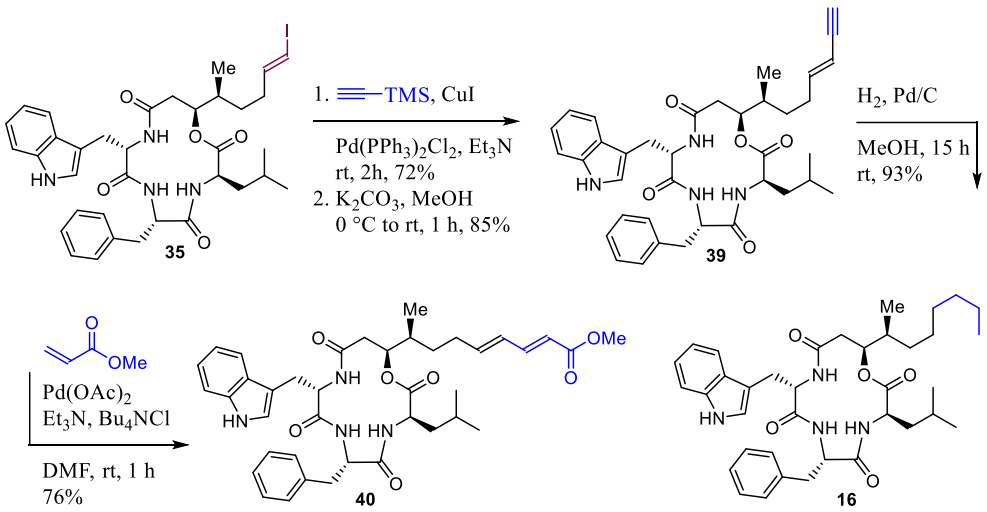
The synthesis of *β*-hydroxy-*γ*-methyl aliphatic acid component is shown in Scheme 3. The approaches to the cyclic intermediate **35** are not only efficient, but also extremely adaptable. Starting from commercially available Boc protected *L*-tryptophan (**31**), alcohol **32** was constructed which was then esterified with acid **33a** using Shinna condition to provide the advance stage precursor **34** which finally was subjected to macrolactamization to obtain intermediate **35** after suitable modifications. On the other hand, alcohol **36** was esterified with acid **33b** to yield ester **37** which was transmuted to compound **35** via the precursor **38** following macrolactamization. Notably, the macrolactamization via intermediate **38** (route II) was found to be more efficient compare to intermediate **34** (route I) as the esterification was found to be the determining step.

Scheme 3. *Synthesis of Vinyl Iodide* ***9*** *Using a Macrolactamization Approach.*



The completion of the total synthesis of beauveamide A (**16**) is depicted in Scheme 4. Vinyl iodide **35** was subjected to Sonogashira coupling using TMS-acetylene in the presence of $Pd(Ph_3P)_2Cl_2/Et_3N$ and treated further with K_2CO_3 in MeOH to yield compound **39**. It was finally

Scheme 4. *Completion of the Total Synthesis of Beauveamide A (**1**) and Its Congeners Using Late-Stage Functionalization Approach.*

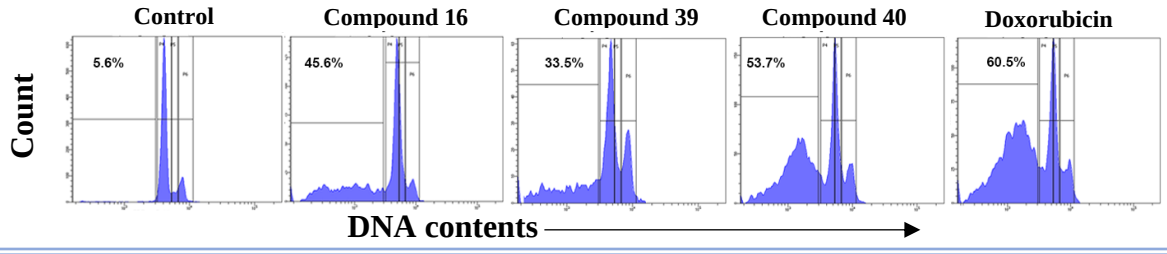


hydrogenated to achieve compound **16**. In order to modify the HMDA segment, vinyl iodide **35** was reacted with methyl acrylate in the presence of $Pd(Ph_3P)_2Cl_2/Et_3N/Bu_4NCl$ to obtain compound **40**.

The in vitro cytotoxic effects of synthesized compound **16** and its congeners **39** and **40** were determined by the MTT assay using HeLa and MDA-MB-231 cancer cell lines. The results suggested that the cytotoxicity of compound **40** was better than that of beauveamide A (**1**), whereas compound **39** was found to be the least active. This study will eventually explore beauveamide A variants by coupling different partners with key vinyl intermediate **35** to understand their structure-activity relationships.

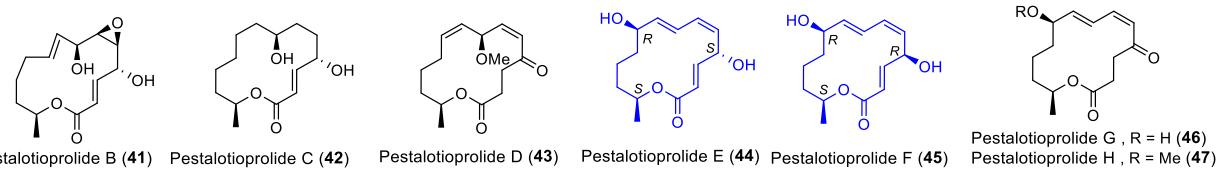
(***Org. Lett.*** **2022**, 24, 39, 7113–7117).

Figure 4: *Compounds reduce cancer cell viability by triggering apoptosis. The apoptotic cell population was measured by flow cytometry analysis. MDA-MB-231 cells were treated with 25µM of Compounds (**16**, **39**, and **40**) for 48 h, stained with PI, and cell cycle analysis was performed in the flow cytometer. The percentage of apoptotic cells is mentioned.*



(iii) Macrolides occurring in nature have attracted the scientific community with their enormous structural diversities and extensive biological activities. In 2016, the research groups of Liu and Proksch isolated pestalotioprolides B-H (**41**-**47**; Figure 5), a 14-membered series of macrolides

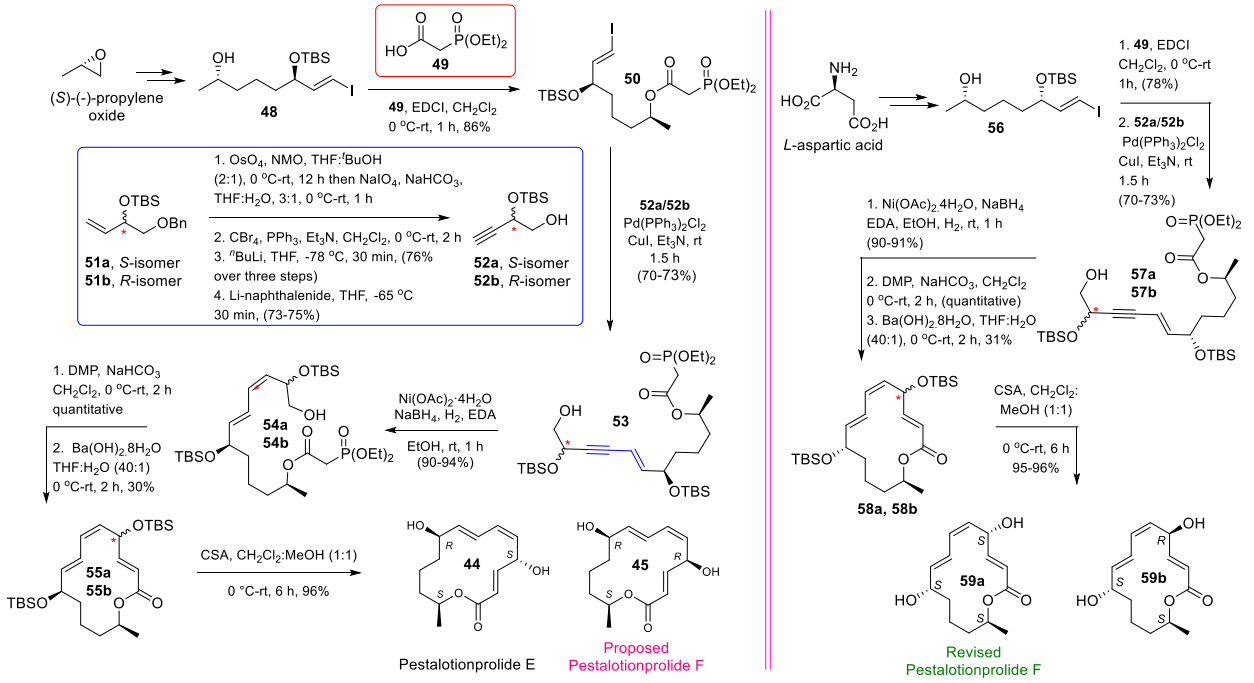
***Figure 5**: Chemical Structures of Pestalotioprolides (B-H).*



with promising architectural features, from *Pestalotiopsis microspora*, an endophytic fungus collected from fresh fruits of the mangrove plant, *Drepanocarpus lunatus*, found in Cameroon. Many of them have shown excellent to moderate cytotoxicity against murine lymphoma (L5178Y) and human ovarian cancer (A2780) cell lines. Their attractive structural features, significant bioactivity, and limited natural abundance make them excellent targets for synthesis. Pestalotioprolides E and F were cytotoxic to the murine lymphoma cell line (L5178Y), with IC50 values of 3.4 and 3.9 µM, respectively, whereas pestalotioprolide E shown promising activity against the human ovarian cancer cell line (A2780), with an IC_{50} value of 1.2 µM. Pestalotioprolides E and F are structurally comprised of three stereogenic centres, two of which are hydroxylated, a conjugated diene with *E*, *Z*-geometry, and another *E*-olefin conjugated with the lactone carbonyl. The presence of a conjugated diene and an olefin separated by a hydroxylated center in a 14-membered macrocycle is unusual, posing an intimidating challenge to the synthetic community.

We have designed the total synthesis of pestalotioprolides E and F (Scheme 5) in a context of rarely used intramolecular HWE reaction to construct a 14-membered ring. We used this strategy as one of the crucial stepping stone in the macrocyclization process to converge our synthesis and successfully incorporate carbonyl-conjugated olefins and conjugated dienes with diverse geometries. Our initial synthesis started from compound **48**, which coupled at a very early stage with the two enantiomeric compounds **52a** and **52b** and finally led to the two diastereomeric compounds **44** and **45**. While, compound **44** turned out to be identical to the reported pestalotioprolide E, the synthesized compound **45** showed several differences with the literature report of pestalotioprolide F, prompting us to synthesize two more isomers (**59a** and **59b**) starting from *L*-aspartic acid (Scheme 5). Delightfully, compound **59a** was found to be in good agreement

***Scheme 5**: Total Synthesis of Pestalotioprolides E and F.*



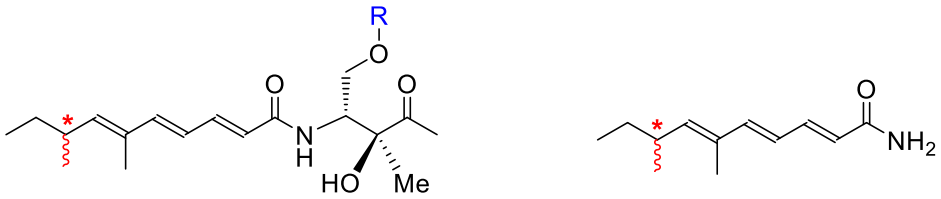
with the data reported for pestalothioprolide F, unquestionably prompted the revision of the actual structure of pestalothioprolide F to compound **59a**.

Further, our recent study (*unpublished*) reveled the unexplored anticancer activity of our synthesized macrolide, Pestalotioprolide E (**44**) and its underlying signaling cascade. This study provides experimental evidence that Pestalotioprolide E (**44**) exhibits a potential anticancer effect on a triple-negative breast cancer cell line MDA-MB-231 by inactivating TRXR1, a prime target in cancer therapy. Thioredoxin reductase functions as a key player in the cancer-associated redox-sensitive signaling cascades that regulate cell growth, proliferation, apoptosis, and metastasis. Pestalotioprolide E can directly interact with TRXR1 and inhibits its enzymatic activity. This inhibition leads to the induction of apoptosis via TRX1/ASK1/p38 MAPK death signaling cascade and causes retardation of metastasis through modulating several metastasis-associated proteins (VEGF, MMP-2, MMP-9, E-cadherin, N-cadherin) in MDA-MB-231 cells. This study is thus bears a great pharmaceutical significance as it discloses the mechanism underlying the biological activity of Pestalotioprolide E, and aids in understanding its action in cancer therapy.

(**Org. Lett., 2018**, *20*, 4606–4609).

(iv) Bipolamides A and B (Figure 6) were discovered from the endophytic fungus *Bipolaris* sp. MU34 from Thai medicinal plants in the year 2014. Among them, Bipolamide A (**60**) has moderate antifungal activity against Cladosporium cladosporioides FERMS-9, Cladosporium cucumerinum NBRC 6370, Saccharomyces cerevisiae ATCC 9804, Aspergillus niger ATCC 6275 and Rhisopus oryzae ATCC 10404, with Minimum inhibitory concentration (MIC) values of 16, 32, 32, 64 and 64 µg/mL, respectively. The biological activity with unique structural features and unknown chiral centre at C-8 makes the molecule pharmacologically more important. The amine counterpart in bipolamide A (**60**) (Figure 6) has a rarely found branched five-carbon acyloin moiety consisting

***Figure 6**: Reported structure of Bipolamide A (60), Bipolamide B (61), Bipolamide A acetate (62).*



R = H; Bipolamide A (**60**)
R= Ac; Bipolamide A acetate(**62**)

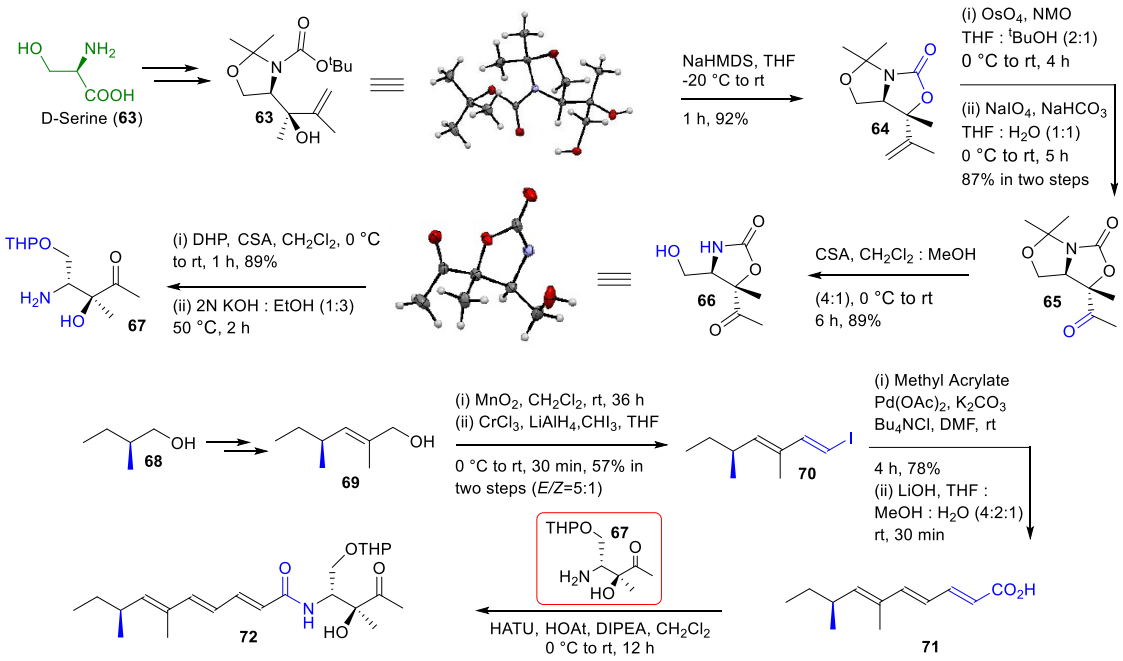
Bipolamide B (**61**)

of two stereocenters among which one is tertiary. Bipolamide A was found to be unstable. Thus, a relatively more stable acetate analogue of bipolamide A (**60**) was prepared by the isolation group during the structural analysis of the parent molecule. The stereochemistry of the C-8 methyl center remained unassigned when reported which prompted us to synthesize two possible stereotriads of bipolamide A acetate to compare their spectroscopic data with those of the reported data to solve the structural riddle of bipolamide A (**60**).

Initial synthesis using a linear approach turned out to be unproductive because, despite of numerous attempts to get compound **72**, most of the cases the yields were relatively poor (below 30% in two stages). The HATU/ HOAt condition had a somewhat better outcome (36% in two stages) compare to the others. The poor yield of amidation reaction might be attributed to the acid having extended conjugation. This finding may be tested with a less conjugated acid, thus we looked for another circumstance in which the amidation could be evaluated with a less conjugated acid.

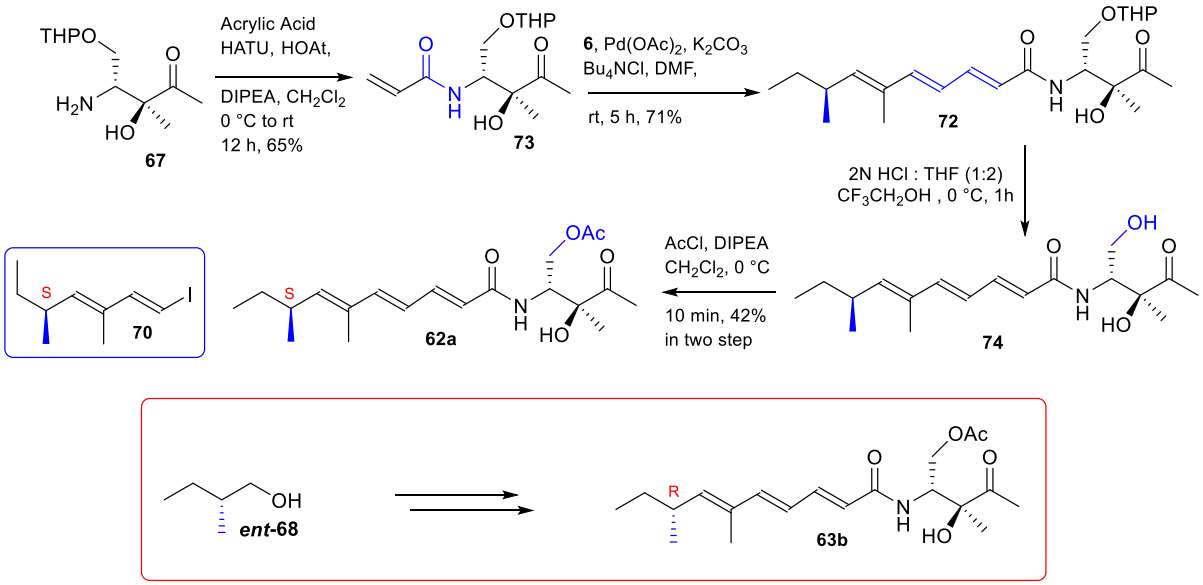
The completion of total synthesis of bipolamide A acetate is shown in Scheme 7. Here, amine **67** was coupled with acrylic acid from the very initial steps. Delightfully, the amidation

***Scheme 6**: Effort toward the Synthesis of Bipolamide A Acetate using a linear approach.*



reaction was far more efficient compared to the earlier case. Compound **73** was then coupled with vinyl iodide **70** mixed with its inseparable minor Z-counterpart in a convergent fashion using Heck reaction to obtain compound **72** along with its corresponding *Z*-isomer which were separated easily by silica gel column chromatography. Deprotection of THP ether of compound **72** was tricky as in most of the cases either the starting material decomposed or the required product formed in trace amount. However, it was a great relief to be able to access compound **74** using 2N HCl : THF (1 : 2) in CF_3CH_2OH. Notably, compound **74** was not so stable to allow further characterization. Thus, it was immediately transformed to its acetate analogue **62a**. Similarly, starting from vinyl iodide ***ent*-68** another probable diastereomer **62b** was synthesized mimicking the route utilized to synthesize compound **62a**. Both the ^{1}H and ^{13}C NMR data of synthesized compounds **62a** and **62b** were analyzed and were found to be in good agreement with the reported data. No such considerable mismatch was observed in any of the cases. However, comparison of optical rotation of synthesized compounds **62a** {observed $[\alpha]^{25}_{D}$ = +44.3 (*c* 0.05, MeOH)} and **62b** {observed $[\alpha]^{25}_{D}$ = -41.9 (*c* 0.05, MeOH)} with the literature data {reported $[\alpha]^{27}_{D}$ = -46.0 (*c*0.05, MeOH)} clearly confirmed compound **62b** as the actual structure of bipolamide A acetate. Hence, the unassigned C-8 center of isolated bipolamide A has been assigned successfully to be in the *R*-

Scheme 7: *Alternative Approach for the Synthesis of Bipolamide A Acetate*.



configuration along with reconfirmation of absolute stereochemistry of the other asymmetric centers.

Although, initial antimicrobial activity assay did not clearly disclose the role of these secondary metabolites to the fungus or the host plant due to the instability of the molecule itself. However, they could be a potential precursor(s) for synthesizing the signaling molecules to regulate the metabolism in the fungus and/or host plant as similar precedent exists in the literature where some fatty acid amides are known to serve an important role in chemical signaling in plants and animals for physiological processes. Thus, our ongoing study on the development of synthetic routes for the quick access of various analogues and their subsequent biological evaluation may have a crucial impact to medicinal chemistry world.

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