Antineoplastic agents. Total synthesis of Dolastatin 16

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Introduction

Education:
Ph.D., Wayne State University, 1956

Research Interests:
• chemistry of natural products (peptides, nucleotides, and steroids)
• cancer chemotherapy (anticancer agents from arthropods, marine animals and plants)
• total synthesis of natural products.

Robert Pettit's group is organized to provide the specialized training necessary to undertake problems concerned with the discovery of anticancer substances for the treatment of cancer. Among various activities, they are pursuing a unique program concerned with isolation, structural identification and synthesis of naturally occurring anticancer agents from marine animals, plants, and arthropods.

https://chemistry.asu.edu/faculty/r_pettit.asp
Nature Sources of Dolastatin 16

Sea hare *Dolabella auricularia* and Madagascan cyanobacterium *Lyngbya majuscula*

**Dolastatin 16** from sea hare *Dolabella auricularia* is an inhibitor of cancer cell growth. ($GI_{50} = 10^{-3} – 10^{-4} \mu g/ml$)

**Dolastatin 16** also has antifouling activity ($EC_{50} = 0.003 \mu g/ml$) against the larvae of the barnacle *Balanus amphitrite*, as well as low toxicity ($LC_{50} = 20\mu g/ml$)

**Dolastatin 16** was originally isolated (in $3.1 \times 10^{-7}\%$ yield) as an amorphous powder. Very slow (over three years) crystal formation from acetonitrile and water provided X-ray quality crystals.

Synthesis of the Dolamethylleuine (Dml) as its Z-protected synthon\(^3\)

\[
\text{Dolastatin 16 (1)}
\]

\[
\text{Dolamethylleuine (Dml)}
\]

\[
\text{Dolaphenvaline (Dpv)}
\]

\[
\text{X-ray structure of dolastatin 16 (1), a β-amino acid, and dolaphenvaline (Dpv), a β-amino acid, and dolaphenvaline (Dpv), a β-amino acid, and dolaphenvaline (Dpv). As part of the Eistert reaction followed by a Wolff rearrangement,} \]

\[
\text{Z-Dolamethylleuine (8) was later reported by Scheuer.}
\]

\[
\text{As part of the} \]

\[
\text{Deprotection and triethylsilane (TES) in DCM provided} \]

\[
\text{Overall yield of 8 = 13%}
\]

Synthesis of the Dolaphenvaline (Dpv)\textsuperscript{4}

\[ \text{Scheme 1} \]

\[ \text{10} \]

\[ \text{11a, 11b} \]

\[ \text{3a} \]

\[ \text{3b} \]

\[ \text{3c} \]

\[ \text{3d} \]

\[ \text{9 (2S)} \]

\[ \text{12(2R)} \]

\[ \text{a} \text{ (a) Idobenzene, AgNO}_3, \text{Pd(OAc)}_2, \text{MeCN; (b) H}_2, \text{PtO}_2, \text{EtOH; (c) 6 M HCl–AcOH (2:1), 120 °C.} \]

Synthesis of the Dolaphenvaline (Dpv)\(^3\)

![Scheme 2](image)

enantio- and diastereoselective Mannich reactions promoted by ... acid units have been prepared, total synthesis of ... through asymmetric Claisen rearrangement.

Herein, we report the asymmetric synthesis of these unusual amino acid units. While attempted nucleophilic addition of PhMgBr, PhLi, or PhMgBr, ZnCl₂-PMP) group. One-pot protecting group manipulation followed. The synthetic plan for both amino acid units (Scheme 2) through diastereoselective alkylation failed, we eventually found ...)

Enantio- and diastereoselective Mannich reactions promoted by Philinopsis speciosa

In conjunction with our program directed toward a practical approach through asymmetric Claisen rearrangement with iodobenzene and non-diastereoselective hydrogenation.

Scheme 1: Total synthesis of **O**

The synthesis involved a Mizoroki–Heck reaction of **O** with **O**, such as diastereomers. As reported by Hayashi, we developed a concise and scalable synthetic strategy for **O**. Previously syntheses of **O** failed, we eventually found a suitable method for the synthesis.

**Scheme 2.**

1. CAN, H₂SO₄, MeCN, H₂O, rt
2. Boc₂O, Na₂S₂O₃, NaHCO₃, rt

>95% ee, *dr* = >95:5, one-pot 73%

MeO₂C—NH—CO₂Et

-78 °C then rt 78%

**O**

**Scheme 3.**

1. O₃; Me₂S, MeOH
2. PhMgBr, ZnCl₂
3. H₂, Pd(OH)₂/C

**O**

**Scheme 4.**

The organocatalytic Mannich reaction was successfully performed in 93% yield. When the reaction was carried out at ambient temperature with aminosulfone (3.0 equiv.), as shown in Scheme 5, excellent enantioselectivity (99% ee, dr = >95:5) was achieved using the carboxylic acid derived from Philinopsis speciosa.

For gram scale synthesis, 1.0 g (2.6 mmol) of NHTs was converted to NHBoc in 67% yield (dr = 8:1). In following the protocol described by Hayashi, a twofold increase in the catalyst was moderate at room temperature. A twofold increase in the catalyst was used following the protocol described by Hayashi, excellent enantioselectivity (entries 5 and 6).

For convenient gram-scale preparation of 28, as shown in Scheme 5, the 1,2-addition reaction occurred cleanly in a highly stereoselective manner (99% ee, dr = >95:5). The conversion of the aldehyde into the anti-Mannich reaction catalyzed by 2-Methyl-2-butene was exclusively formed as a single stereoisomer in 70% yield. We envisioned the derivation of 3-M-phthalimido and lactone-mediated synthesis of 28.

The 1,2-addition reaction occurred cleanly in a highly stereoselective manner (99% ee, dr = >95:5). As reported by Hayashi, although the Mannich reaction proceeded sluggishly in DMF or DMSO (entries 3 and 4), THF was found to be superior to 1,4-dioxane. We then determined by chiral HPLC analysis with 90% yield.

Although the two amino groups failed, we eventually found 3-M-phthalimido was isolated in 85% yield (two steps). Protecting group transformation, generated from methacrolein and lactone, was reported by Hayashi. Pettit also achieved a synthesis of 28. When the reaction was carried out at ambient temperature, the organocatalytic Mannich reaction was successfully performed in 93% yield. The overall yield of 28 was 48% (5 steps).
Retrosynthetic analysis of Dolastatin 16

Figure 2. Retrosynthetic analysis for dolastatin 16 (1).

Scheme 1. Synthesis of Intermediate 7

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B
Synthesis of Intermediate 7 of Dolastatin 16

The presence of silver(I) oxide in order to obtain compound 15 in 51% yield. Compound 15 was hydrolyzed in the presence of potassium hydroxide to obtain acid 16 in 88% yield. Then, compound 16 was treated with L-proline tert-butyl ester in the presence of the peptide coupling reagent PyBroP and diisopropylethylamine (DIPEA) to obtain compound 8 as a mixture of rotamers in 82% yield. Compound 8 was deprotected with trifluoroacetic acid and coupled to intermediate 7 using 2-methyl-6-nitrobenzoic anhydride (MNBA) in the presence of 4-dimethylaminopyridine (DMAP) and triethylamine to afford compound 17 as a complex mixture of rotamers in 81% yield. Finally, compound 17 was treated with trifluoroacetic acid (TFA) to obtain intermediate 5 again as a mixture of rotamers in quantitative yield. The synthesis of the hydrochloric salt of dolaphenvaline was accomplished in six steps following a strategy developed by Li.
Synthesis of Intermediate 5

**Scheme 2. Synthesis of Intermediate 5**

1. **OH** \(\xrightarrow{\text{BnBr, Ag}_2\text{O}}\)** O\(\text{Bn}\) \(\xrightarrow{\text{KOH}}\)** O\(\text{Bn}\) \(\xrightarrow{\text{PyBroP, DIPEA, H-Pro-OtBu+HCl}}\)** BnO\(\text{O} \text{N}\) CO\(\text{O}\) CO\(\text{O}\)

2. 1. **TFA** \(\xrightarrow{\text{anh. DCM}}\)** 81%  
   2. **7, MNBA, DMAP, TEA** \(\xrightarrow{\text{anh. DCM}}\)** 81%  

**Scheme 3. Synthesis of Dolaphenvaline · HCl (9)**

1. **OH** \(\xrightarrow{\text{BnBr, Ag}_2\text{O}}\)** O\(\text{Bn}\) \(\xrightarrow{\text{KOH}}\)** O\(\text{Bn}\) \(\xrightarrow{\text{PyBroP, DIPEA, H-Pro-OtBu+HCl}}\)** BnO\(\text{O} \text{N}\) CO\(\text{O}\) CO\(\text{O}\)

2. **OH** \(\xrightarrow{\text{TFA}}\)** \(\text{O} \text{N}\) CO\(\text{O}\) CO\(\text{O}\)

**Scheme 4. Synthesis of Dolastatin 16 (1)**

1. **OH** \(\xrightarrow{\text{BnBr, Ag}_2\text{O}}\)** O\(\text{Bn}\) \(\xrightarrow{\text{KOH}}\)** O\(\text{Bn}\) \(\xrightarrow{\text{PyBroP, DIPEA, H-Pro-OtBu+HCl}}\)** BnO\(\text{O} \text{N}\) CO\(\text{O}\) CO\(\text{O}\)

2. **OH** \(\xrightarrow{\text{TFA}}\)** \(\text{O} \text{N}\) CO\(\text{O}\) CO\(\text{O}\)
Synthesis of Intermediate 9

1. Pivaloyl chloride, TEA.  
2. n-BuLi, 
R-(-)-4-Ph-2-oxazolidinone  
anh. THF  
94% 

18

CuBr-Me₂S, BnMgCl  
anh. THF/ anh DMS

20

LiOH, H₂O₂  
THF/water

N₃

19

NBS  
67%, two steps

67%, two steps

1. Pd/C, H₂ (36 psi), AcOH/water  
2. 6 M HCl  
78%, three steps

9

Dolaphenvaline · HCl
Synthesis of Dolastatin 16

In 67% yield. Compound 24 was then hydrolyzed using LiOH followed by treatment with benzyl bromide in the presence of triethylamine to obtain compound 6 in 56% yield. Then, compound 6 was treated with TFA to deprotect the amino group. The crude product was next treated with compound 5 in the presence of HBTU and triethylamine to obtain compound 4 as a mixture of rotamers in 87% yield over two steps. For the last step, compound 4 was subjected to hydrogenolysis to remove the two benzyl groups and then treated with MNBA in the presence of DMAP and triethylamine under high dilution to afford dolastatin 16 (1) in 22% yield. The synthetic dolastatin 16 was found to be identical to the natural product as compared by HPLC, NMR (400 MHz), optical rotation, HRMS, and X-ray crystallography data (Figure 3). The X-ray crystal structure observed for the synthetic dolastatin 16 showed the same stereochemistry as the natural dolastatin 16; however, there are small differences in bond angles due to the solvent used for crystallization.

Biological evaluation of the synthetic dolastatin 16 against a small panel of cancer cell lines showed a surprising lack of cancer cell growth inhibition (GI₅₀ > 10 μg/mL) as compared to the natural counterpart, which consistently led to GI₅₀ 0.0012−0.00096 μg/mL cancer cell growth inhibition against a minipanel of human cancer cell lines. The results of this analysis suggest a conformational change in the synthetic specimen or presence of a chemically undetected compound in the sample that was isolated from the natural source in 1997. Previously, it was observed that certain cyclic depsipeptides could carry traces of compounds too small to be detected by NMR or chromatographic techniques responsible for the biological activity.

In 2011, dolastatin 16 was also isolated from the cyanobacterium Symploca cf. hydnoides by Luesch and colleagues. The activity of dolastatin 16 isolated from this particular organism was greatly lower (IC₅₀'s of 69 and 51 μg/mL for the HT-29 and HeLa cell lines, respectively) as compared to the specimen isolated from D. auricularia. Recently, it was shown that the activity of phakellistatin 2, a cyclic peptide also containing proline residues in its sequence, exhibited different cancer cell growth inhibition depending on if methanol or dimethyl sulfoxide was used for the bioassay. These findings suggest that conformational changes, due to the solvent used, can have a big impact on the biological activity of certain macrocycles containing proline residues and possibly N-alkylated amino acids. In fact, NMR data (not shown) were also collected in deuterated solvents such as methanol and dimethyl sulfoxide, and the presence of two or more conformers was observed. Following the logic in the findings mentioned above, the synthetic dolastatin 16 was also evaluated in methanol; unfortunately, no activity was observed (Table 1). The results shown for the natural sample of dolastatin 16 require some background. Table 1 data (current, 2014) for the natural dolastatin 16 were on a very small sample from the remaining ∼100 μg from the original isolation (3.1 mg, 10⁻⁷% yield) from 1000 kg (wet weight) of the sea hare D. auricularia that we collected in Papua New Guinea in 1983 and reported following 14 years of research by 1997.
could carry traces of compounds too small to be detected by.

Previously, it was observed that certain cyclic depsipeptides—the sample that was isolated from the natural source in 1997. Specimen or presence of a chemically undetected compound in analysis suggest a conformational change in the synthetic specimen.

Scheme 4. Synthesis of Dolastatin 16

Journal of Natural Products

Triethylamine to obtain compound 6 followed by treatment with benzyl bromide in the presence of DMAP and triethylamine under high dilution to remove the two benzyl groups and then treated with MNBA in the last step. Compound 5 was subjected to hydrogenolysis to yield. The strategy followed here for the synthesis of Dml is stereoselective as compared to the synthesis performed by Kimura et al. using an Evan B type chiral auxiliary to control the stereochemistry (Scheme 3).

The synthetic dolastatin 16 was found to be identical to the dolastatin 16 synthesized in seven steps before we used this sample, it was shown for the natural sample of dolastatin 16 require some unactivities were observed (Table 1). The results of this biological evaluation of the synthetic dolastatin 16 against a small panel of cancer cell lines showed a surprising lack of activity.

The results of this were on a very small sample from the remaining 14 years of research by 1997. Unfortunately, no activity was observed (Table 1). The results of this biological evaluation of the synthetic dolastatin 16 was also evaluated in methanol; sulfoxide, and the presence of two or more conformers was collected in deuterated solvents such as methanol and dimethyl sulfoxide, and the presence of two or more conformers was collected in deuterated solvents such as methanol and dimethyl sulfoxide. In fact, NMR data (not shown) were also compared to the specimen isolated from D. auricularia.

These findings suggest that conformational changes, due to the particular organism was greatly lower (IC_{50} μg/mL cancer cell growth inhibition against μg/mL) as compared

1 \text{.} Pd(OH)_{2}/C, H_{2}, \text{EtOH}

2 \text{.} MNBA, DMAP, TEA, anh. Tol

87%
After purification, a reduced activity was observed in some cancer cell lines as growth inhibitor, except for DU-145 (prostate), as compared to the previous results obtained for the original dolastatin 16. Although the utility of the synthetic dolastatin 16 for cancer cell growth inhibition is disappointing, we will begin an evaluation against other medical indications and the potential of SAR modifications especially involving the proline unit.

In recent years other proline-rich cyclodepsipeptides, analogues of dolastatin 16, have been isolated from different organisms. Kulokekahilide-1 was isolated from the cephalaspidean mollusk Philinopsis speciosa collected from Shark's Cove, Pupukea, O‘ahu, in 2002. Homodolastatin 16 and pitiprolamide were later isolated by different research groups (in 2003 and 2011, respectively) from the marine cyanobacterium Lyngbya majuscula collected from different parts of the

**Human Cancer Cell Growth Evaluation of Natural and Synthetic Dolastatin 16, GI₅₀ (μg/mL)**

<table>
<thead>
<tr>
<th>compound</th>
<th>solvent</th>
<th>BXPC-3</th>
<th>MCF-7</th>
<th>SF-268</th>
<th>NCI-H460</th>
<th>KM20L2</th>
<th>DU-145</th>
</tr>
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<tbody>
<tr>
<td>dolastatin 16 (natural)</td>
<td>DMSO</td>
<td>0.050</td>
<td>0.027</td>
<td>0.016</td>
<td>0.270</td>
<td>0.013</td>
<td>0.009</td>
</tr>
<tr>
<td>dolastatin 16 (synthetic)</td>
<td>DMSO</td>
<td>&gt;10</td>
<td>&gt;10</td>
<td>&gt;10</td>
<td>&gt;10</td>
<td>&gt;10</td>
<td>&gt;10</td>
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<tr>
<td></td>
<td>MeOH</td>
<td>&gt;10</td>
<td>&gt;10</td>
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</tr>
</tbody>
</table>

*Cell lines in order: pancreas (BXPC-3); breast (MCF-7); CNS (SF-268); lung (NCI-H460); colon (KM20L2); prostate (DU-145).*

**Table 1. Human Cancer Cell Growth Evaluation of Natural and Synthetic Dolastatin 16, GI₅₀ (μg/mL)**

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<td>&gt;10</td>
<td>&gt;10</td>
<td>&gt;10</td>
</tr>
<tr>
<td></td>
<td>MeOH</td>
<td>&gt;10</td>
<td>&gt;10</td>
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</tbody>
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*Cancer cell lines in order: pancreas (BXPC-3); breast (MCF-7); CNS (SF-268); lung (NCI-H460); colon (KM20L2); prostate (DU-145).*
Thank you for your attention!