Natural anti-cancer: The Occurrence, Bioactivity, Biosynthesis and Synthesis of Pancratistatin

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Introduction

Natural anti-cancer: The Occurrence, Bioactivity, Biosynthesis and Synthesis of Pancratistatin: Pancratistatin (PST) is a natural compound that was initially extracted form Spider Lily[1], a Hawaiian native plant, belonging to the family of Amaryllidaceae[2] (AMD). PST has shown some strong anti cancer activities[3] by displaying potent toxicity against human tumor cells[4]. Another distinct feature of PST as an anti cancer secondary metabolite is its ability in inducing apoptosis in cancer cells by targeting their mitochondria whilst leaving the normal cells unaffected[5].

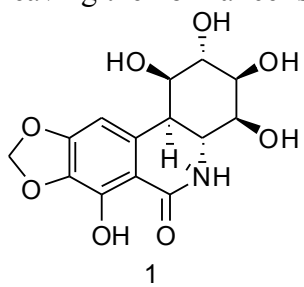

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Figure 1. Pancratistatin

Occurrence

Occurrence: Pancratistatin occurs naturally in Hawaiian Spider Lily, a flowering plant within the *Amaryllidaceae* family. Pancratistatin is mostly found in the bulb tissues of Spider Lillies and it has been shown that the enrichment of atmospheric CO2 can enhance the production of antiviral secondary metabolites, including Pancratistatin, in these plants[6]. Pancratistatin can be isolated from the tropical bulbs of *Hymenocallis littoralis in* the order of 100 to 150 mg/kg when bulbs are obtained from the wild type in Hawaii. However, the compound has to be commercially extracted from field- and greenhouse-grown bulbs or from tissue cultures cultivated, for example, in Arizona, which generate lower levels of Pancratistatin (a maximum of 22 mg/kg) even in the peak month of October. After October, when the bulb becomes dormant, levels of Pancratistatin drop to only 4 mg/kg by May. Field-grown bulbs, which show monthly changes in Pancratistatin content, generate somewhat smaller amounts (2–5 mg/kg) compared to those grown in greenhouse cultivated over the same period[7].

There are about fourty different Spider Lily species worldwide and they are mainly native to Andes of South America.

Schoals Spider Lilly (Source: http://sherpaguides.com/georgia/flint_river/wildnotes/graphics/shoals_spider_lily.jpg)

Spider Lily (http://www.timothyandtonya.com/images/SpiderLily.jpg)

II. Biological Activity

The medicinal and toxic properties of *Amaryllidaceae* family were first discovered by the Greeks. They used the oil from ***Narcissus*** species for the treatment of cancer[8]. Consequently, efforts have been made to isolate the active ingredients responsible for this antitumor activity. Some 48 alkaloids bearing a variety of carbon skeletons have been isolated from ***Narcissus*** species[9]. One group of these alkaloids does not contain basic nitrogen atoms and is represented by isoquinolinone structure. The most widely known compounds of this group are narciclasine, lycoricidine and Pancratistatin[10]. The most frequently used term in the literature to describe this group of alkaloids is the isocarbostyrils [11]. All these natural products have demonstrated potent ***in vitro*** cytotoxicity against cancer cell lines and potent ***in vivo*** antitumor activity Therefore, this family as a whole seems of interest as a potential source of new lead structures for the development of a future generation of anticancer drugs **[12].**

PST displays selective potent toxicity against human tumour cells by showing a strong desire to attack the Mitochondria of the cancer cells and having no influence on normal cells. Studies have shown that Pancratistatin rapidly and efficiently induces apoptosis (programmed cell death) in various types of cancer cell lines, including breast, colon, prostate, neuroblastoma, melanoma and leukemia. Most importantly, when Pancratistatin was tested for toxicity on peripheral white blood cells from healthy volunteers, there was little or no demonstrable effect on their viability and nuclear morphol, indicating the relative specificity of this compd. for cancer cells.

Some of the recent insights regarding the mode of action of PST include inhibition of the cell cycle from G_0/G_1 to S phase and the powerful antiparasite activity[13]. As mentioned previously, Pancratistatin, unlike other anticancer drugs, discerns between the normal and cancerous cells. Pancratistatin does not cause DNA double-strand breaks or DNA damage prior to the execution phase of apoptosis in cancer cells. Parallel experimentation with VP-16, a currently used medication for cancer treatment, indicated that VP-16 causes substantial DNA damage in normal non-cancerous blood cells, while Pancratistatin does not cause any DNA double-strand breaks or DNA damage in non-cancerous cells. Pancratistatin induces apoptosis in cancer cells using non-genomic targets, and more importantly does not seem to have any affect non-cancerous cells, presents a significant platform to develop non-toxic anticancer therapies[14].

The capability of Pancratistatin to selectively induce apoptosis in cancer cells is an exciting finding and makes it a suitable anti-cancer agent. Since Pancratistatin shows little structural similarity to any DNA intercalating drug or to paclitaxel derivatives, it appears to be non-genotoxic. Also, Pancratistatin may act upon target while allowing selective induction of apoptosis in cancer cells[15]. Recent studies have also shown that the bulbs of Pancratium contain a new phenanthridone biosynthetic product designated Pancratistatin that proved to be effective (38-106% life extension at 0.75-12.5 mg/kg dose levels) against the murine P-388 lymphocytic leukemia. Pancratistatin also markedly inhibited (ED50, 0.01 microgram/ml) growth of the P-388 in vitro cell line and in vivo murine M-5076 ovary sarcoma (53-84% life extension at 0.38-3.0 mg/kg) [16].

III. Biosynthesis:

Although there may not be a precise elucidation of Pancratistatin biological synthesis, there have been speculations on BIOSYNTHESIS: Although there may not be a precise elucidation of Pancratistatin biological synthesis, there have been speculations on biosynthesis of Narciclasine and Lycoricidine that are very similar to Pancratistatin in terms of structure. The biosynthesis is accomplished via synthesized from *O*-methylnorbelladine **4** by *para-para* phenol coupling to obtain vittatine **5** as an intermediate Subsequent elimination of two carbon atoms and hydroxylations of compound **5** then leads to narciclasine [17].

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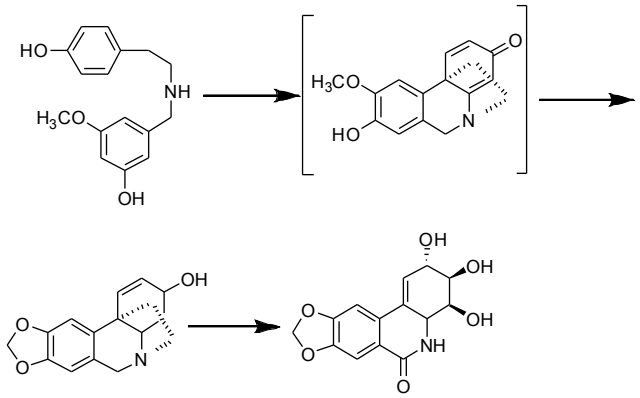


Figure 2. Pancratistatin-like biosynthesis using Narciclasine as a model.

IV. Synthesis :

Total Synthesis:

The first total synthesis of racemic (+/-) Pancratistatin was proposed by Samuel Danishefsky and Joung Yon Lee, which involved a very complex and long (40 steps) total synthesis. According to both Danishefsky and Joung, there were several weak steps in this synthesis that gave rise to a disappointing low synthetic yield. Amongst the most challenging issues, the Moffatt transposition and theorthoamide problem, which required a blocking maneuver to regiospecifically distinguish the C, hydroxyl group for rearrangement were considered to be the severe cases. However, both Danishevsky and Yon Lee stated that their approach towards the PST total synthesis was not out of merit and believed that their work would interest other medicinal scientists to construct a much more practical and efficient way for PST total synthesis [18].

The work of Danishevsky and Joung provided the foundation for another total synthesis of PST, which was propounded by Li,M. in 2006. This method employed a more sophisticated approach starting out with the pinitol **30** that its stereocenters are exactly the same as the ones in the C-ring of Pancratistatin [20].Protection of the diol functions of compound **30** gave compound **31**.The free hydroxyl of this was subsequently substituted by an azide to give **32**. After removal of the silyl function, a cyclic sulfate was installed to obtain product **33**. The Staudinger reaction gave the free amine **34** from azide **33**. The coupling reaction between **34** and **35** gave compound **36** with a moderate yield. Methocymethyl protection of both the amide and the free phenol gave compound **37**. Treatment of this latter product with t-BuLi followed by addition of cerium chloride gave compound **38**. Full deprotection of **38** by BBr_3 and methanol afforded pancratistatin **3** in 12 steps from commercially available pinitol with an overall yield of 2.3% **20**.

a: $TIPDSCl_2$, imidazole, DMAP, DMF, 24%. **b:** DMP, *p*-TsOH, acetone, 81%. **c:** PPh_3, DEAD, CH_3SO_3H, CH_2Cl_2, 0°C to r.t. then NaN_3, DMF, 60°C, 72%. **d:** TBAF, THF, 0°C to r.t., 100%. **e:** $SOCl_2$, Et_3N, CH_2Cl_2, 0°C. **f:** $NaIO_4$, $RuCl_3$, aq CH_3CN, 87% (more than two steps). **g:** PPh_3, aq THF, 0°C to r.t., 94%. **h:** Et_2O, **35**, 0°C, 64%. **i:** K_2CO_3, MOMCl,DMF, 84%. **j:** *t*-BuLi, $CeCl_3$, ultrasound, THF, -78°C to r.t., 72%. **k:** BBr_3, CH_2Cl_2, -78°C to 0°C, 1 hour then MeOH, -78°C to 0°C, 2 hours, 52%.

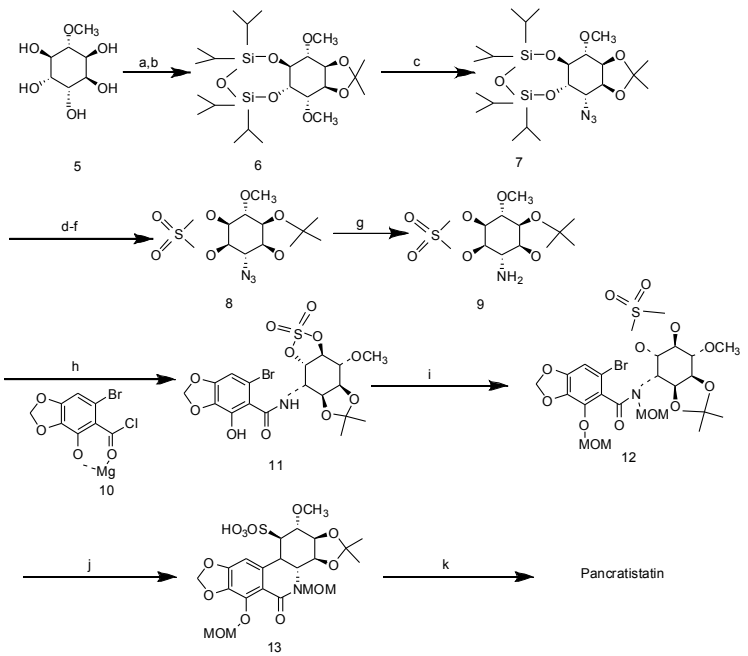


Figure 3. Total Synthesis of racemic Pancratistatin

Stereocontrolled Total Synthesis of Pancratistatin[20]

Abstract:

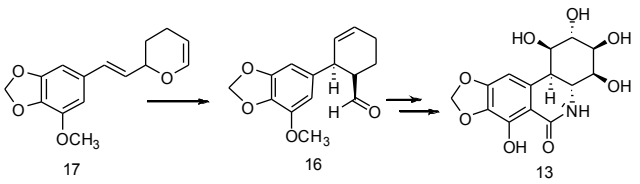
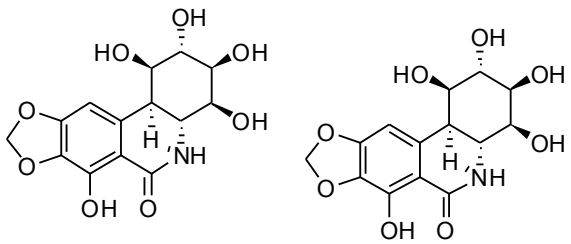


Figure 4. The abstract of the Stereocontrolled synthesis of Pancratistatin



(+)-pancratistatin 13 (+)-narciclasine 14

Figure 5. Pancratistatin and Narciclasine

Scheme 1:

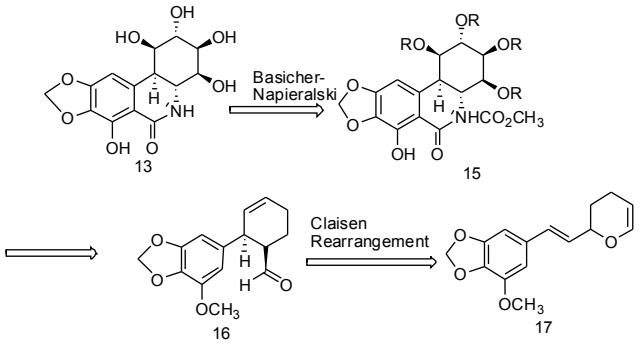
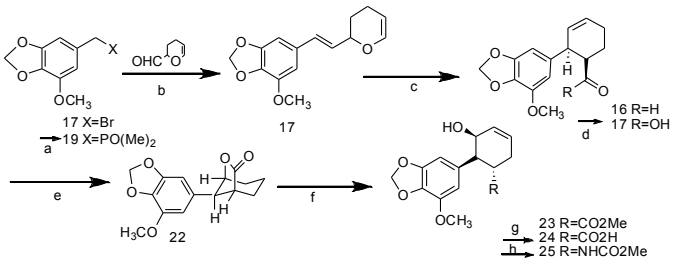
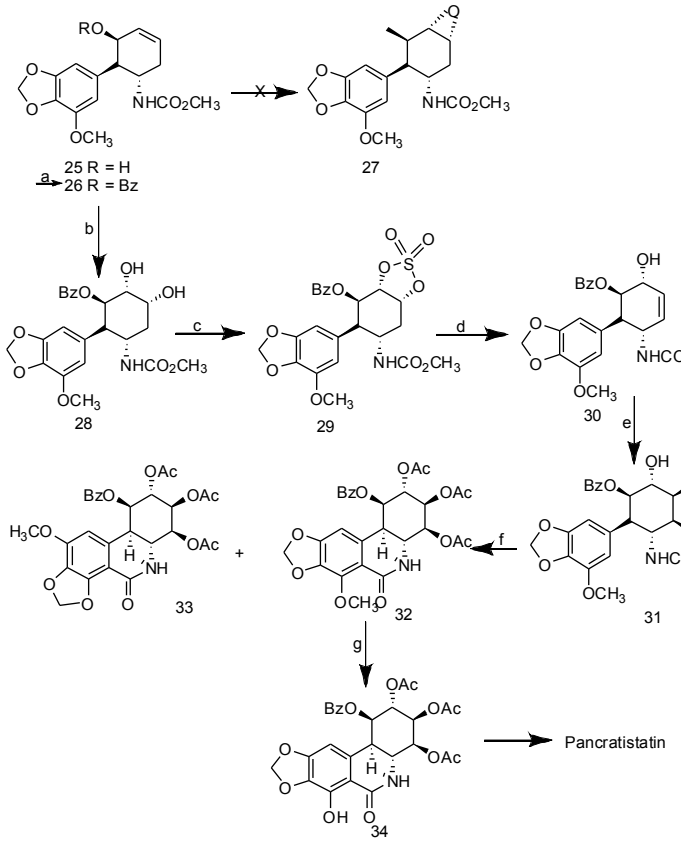


Figure 6. Streocontrolled synthesis of pancratistatin

Scheme 2:



Scheme 3:



A very recent approach to a stereocontrolled Pancratistatin synthesis was accomplished by Sanghee Kim from the National University of Seoul, in which claisen rearrangement of dihydropyranethlyene and a cyclic sulfate elimination reaction were employed[21]. This reaction has proven to be very highly efficient as it produced an 83% overall synthetis yield. (Proved by H and ^{13}C NMR).

The B ring of the phenanthridone (three membered nitrogen hetrocyclic ring) is formed using the Bischler-Napieralski reaction. The n precursor **3** with its stereocenters in the C ring is stereoselectively synthesized from the *cis*-disubstituted cyclohexene **4**. The presence of unsaturated carbonyl in compound **4** suggested the use of a Claisen rearrangement of 3,4-dihydro-2*H*-pyranylethylene[21].

The synthesis starts with the treatment of 6 with excess trimethyl phosphate. This reaction provides phosphate 7 in 97% yield. Using Honer-Wadsworth-Emmons reaction between 7 ands acrolein dimmer 8 in the presence of LHMDS in THF forms (E)-olefin 5 with very high stereoselectivity in 60% yield. Only less than 1% of (Z)-olefin was detected in the final product. The Claisen rearrangement of dihydropyranethylene forms the cis-distributed cyclohexene as a single isomer in 78% yield.

The next step of the synthesis involves the oxidation of aldehyde of compound 4 using NaClO2 to the corresponding carboxylic acid 9 in 90% yield. Iodolactonization of 9 and subsequent treatment with DBU in refluxing benzene gives rise to the bicyclic lacytone in 78% yield. Mthanolysis of lactone 10 with NaOMe forms a mixture of hydroxyl ester 11 and its C-4a epimer (Pancratistatin numbering). Saponification of the methyl ester 11 with LiOH was followed by a Curtius rearrangement of the resulting acid 12 with diphenylphosphoryl azide in refluxing toluene to afford isocyanate intermediate, which its treatment with NaOMe/MeOH forms the corresponding carbamate 13 in 82% yield.
The next steps of the synthesis involves the regioselevtive elimination of C-3 hydroxyl group and subsequent unsaturation achieved by cyclic sulfate elimination. Diol 16 needs to be treated with thionyl chloride and further oxidation with RuCl3 provides the cyclic sulfate 17 in 83% yield [22]. Treatment of cyclic sulfate with DBU yields the desired allylic alcohol 18 (67% yield).
Reaction with OsO4 forms the single isomerlization 19 in 88% yield. Peracetylation of 19 (77% yield) accompanied by Banwell's modified Bischler-Napieralski forms the compound 20 with a little amount of isomer 21 (7:1 regioselectivity). The removal of protecting groups with NaOMe/MeOH forms Pancratistatin in 83%.

Conclusion:

Pancratistatin is an extremely important natural secondary metabolite in the category of alkaloids, as it has shown high strong antitumor activities. The factor that makes PST a noble anticancer drug is its high selectivity in differentiating between the tumor and normal cells. The very first total synthesis of PST was introduced fairly recently, in 1989, which implies that there's still room for

further improvements in PST synthesis techniques; it was shown in 2006 by South Korean scientists that a stereoselective synthesis of Pancratistatin could be accomplished with a relatively high yield.

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