

Chemistry, Biology, and Medicinal Potential of Narciclasine and its Congeners

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Chemistry, Biology, and Medicinal Potential of Narciclasine and its Congeners

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1. Introduction

Ornamental flower growers know that placing a cut daffodil (aka narcissus) in a vase with other flowers has a negative effect on the quality of those flowers and significantly shortens their vase life. Furthermore, a common horticultural practice for the cultivation of narcissus flowers involves the introduction of cuts on the bulbs before immersing them into water. The mucilage that leaches out from the cuts is constantly removed by frequent changing of the water, and this leads to sprouting. These observations raise speculation that specific components in the mucilage of the narcissus bulbs may have powerful growth-inhibitory effects. Historical use of narcissus flowers, as well as at least 30 other plants of the Amaryllidaceae family, in folk medicine for the management of cancer¹ speaks volumes to validate this conjecture.

Indeed, powerful anticancer properties of *Narcissus poeticus* L. were already known to the Father of Medicine, Hippocrates of Kos (ca. B.C. 460–370), who recommended a pessary prepared from narcissus oil for the treatment of uterine tumors.² His successors, the ancient Greek physicians Pedanius Dioscorides (ca. A.D. 40–90) and Soranus of Ephesus (A.D. 98–138), continued using this therapy in the first and second centuries A.D.^{3,4} In addition, the topical anticancer uses of extracts from this plant^{5,6} as well as from *Narcissus pseudonarcissus*^{7–9} were recorded in the first century A.D. by the Roman natural philosopher Gaius Plinius Secundus (A.D. 23–79), better known as Pliny the Elder.¹⁰ Even the Bible provides multiple references to the Mediterranean *Narcissus tazetta* L., which has a long history of use against cancer.¹¹ The applications of narcissus oil in cancer management continued in the middle ages in Chinese, North African, Central American, and Arabian medicine.^{1,12} The uses of other genera of the Amaryllidaceae family were also common; for example, *Hymenocallis caribaea* (L. emend Gawler) Herbert was utilized by early European medical practitioners for inflammatory tumors.¹³

More recently, the plants of Amaryllidaceae have been under intense scrutiny for the presence of the specific metabolites responsible for the medicinal properties associated with this plant family. The study began in 1877 with the isolation of alkaloid lycorine from *N. pseudonarcissus*,¹⁴

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Alexander Kornienko was born in the small Arctic town of Vorkuta, Russia, in 1971. He studied polymer chemistry at Mendeleev University in Moscow and then moved to the United States, where he received his Ph.D. in synthetic organic chemistry working with Professor Marc d'Alarcao at Tufts University in 1999. After a 2 year postdoctoral stay with Professor Stephen Hanessian at the University of Montreal, working on the synthesis of novel aminoglycoside antibiotics, he started his independent career as Assistant Professor of Chemistry at New Mexico Tech in 2001 and was promoted to Associate Professor in 2006. His research interests encompass broad areas of synthetic organic, biological, and medicinal chemistry. Projects in his laboratory include the development of practical syntheses of natural products and their analogues, discovery of novel synthetic methodology and rational design, and synthesis and biological evaluation of synthetic compound libraries based on natural product scaffolds.



Dr. Evidente is a professor of organic chemistry. He received his degree in Chemistry in 1975 from the University of Naples Federico II. He worked as a chemist of bioactive natural compounds at the University of Naples Federico II and from 1987 to 1989 at the University of Basilicata, Potenza. Dr. Evidente's research has been mainly devoted to the biosynthesis, biochemistry, chemistry, spectroscopy, and synthesis of bioactive metabolites (alkaloids, phytotoxins, plant growth regulators, antibiotics, mycotoxins, fungicides, phytoalexins, herbicides, proteins, and polysaccharides) produced by phytopathogenic fungi and bacteria and plants. In particular, the stereostructural determination and synthesis of bioactive metabolites and studies on structure–activity relationships and on their action modes were performed by using advanced spectroscopic and chemical methods. Prof. Evidente is a leader of national and international research projects on the isolation and structure determination of bioactive microbial and plant metabolites including lipo- and exopolysaccharides. Some of these have been used to correlate the tridimensional structure in solution with their roles in plant pathogenesis and as tools for the correct classification of the producer micro-organisms.

and since then, more than 100 alkaloids, exhibiting diverse biological activities, have been isolated from the Amaryllidaceae plants.

On the basis of the present scientific evidence, it is likely that isocarbostyryl constituents of the Amaryllidaceae, such as narciclasine, pancratistatin, and their congeners, are the

most important metabolites responsible for the therapeutic benefits of these plants in the folk medical treatment of cancer. Notably, *N. poeticus* L. used by the ancient Greek physicians, as was eluded before, is now known to contain some 0.12 g of narciclasine per kg of fresh bulbs.¹⁵ Continuing along this intriguing path, the focus of the present review is a comprehensive literature survey and discussion of the chemistry and biology of these compounds as specifically relevant to their potential use in medicine. The examination of the synthetic organic chemistry, more specifically the total synthesis efforts inspired by the challenging chemical structures of narciclasine, pancratistatin, and their congeners, will be reduced to a minimum in view of the two very recent excellent reviews published on this subject.^{16,17}

2. Plant Sources and Isolation Methods

2.1. Amaryllidaceae Plants in Traditional Medicine

Plants belonging to the Amaryllidaceae family are herbaceous perennials that grow from bulbs. The family consists of about 60 genera, whose 800 species are widely distributed in several countries around the world. They are also cultivated as ornamental plants for their beautiful flowers and for the production of volatile oil. Amaryllidaceae plants are extensively used in traditional medicine in different countries, and their pharmacological effects are frequently associated with several typical alkaloids that they synthesize. The therapeutic action of a range of wild plants, although not scientifically proven, has been discovered by indigenous people over centuries. Developing countries are often subject to shortages of funds, medical facilities, and newly developed medicine, which make them more dependent on their natural sources. Among these, various African, Asian, and Polynesian communities still use traditional remedies for primary health care.¹⁸

The majority of compounds found in the Amaryllidaceae family are alkaloids, which are uniquely associated with its members. The study of Amaryllidaceae alkaloids began in 1877 with the isolation of lycorine (**3**, Figure 1) from *N. pseudonarcissus*,¹⁴ and the interest around this group of naturally occurring compounds has increased over time because of their effective antitumoral and antiviral activities. Lycorine is a pyrrolo[*de*]phenanthridine ring type alkaloid extracted from different Amaryllidaceae genera, whose structure was elucidated by Nagakawa et al. in 1956.¹⁹ However, because of increasing attention attracted by lycorine in the last few decades and, in particular, the ability of **3** to inhibit ascorbic acid synthesis in vivo,²⁰ the chemical and biological aspects of this interesting alkaloid are being further investigated.²¹

Hundreds of new alkaloids isolated from different parts and in different vegetative phases of ca. 150 species belonging to 36 genera can be grouped into 12 distinct ring types (Table 1). The structures of a representative alkaloid of each ring type are shown in Figures 1, 2, 3, and 7.²² The advances made in the isolation as well as chemical and biological characterizations of such alkaloids have been extensively reviewed.^{5,14,21–28}

The initially discovered analgesic activity exhibited by the Amaryllidaceae alkaloids generated a lot of interest, and it was attributed to their resemblance to morphine and codeine skeletons. In this respect, the alkaloids of the pyrrolo[*de*]phenanthridine group are considered more promising than the alkaloids of galanthamine and 5,10b-ethanophenanthridine

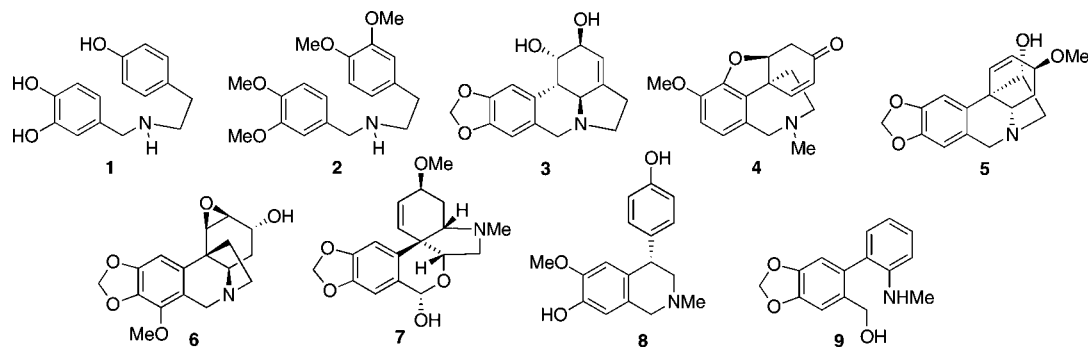


Figure 1. Ring types and representative Amaryllidaceae alkaloids (see Table 1).

Table 1. Ring Types and Representative Amaryllidaceae Alkaloids^{a,b}

	ring type	alkaloid	structure
I	<i>N</i> -(3,4-dioxybenzyl)-4-oxyphenethylamine	norbelladine	1
II	<i>N</i> -(3,4-dioxybenzyl)-3,4-dioxyphenethylamine	rystilline	2
III	pyrrolo[<i>de</i>]phenanthridine	lycorine	3
IV	lycorenine	hippeastrine	10
V	galanthamine	narwedine	4
VI	5,10b-ethanophenanthridine	haemanthamine	5
VII	1,2-epoxy-5,10b-ethanophenanthridine	1,2- β -epoxyambelline	6
VIII	pretazettine	pretazettine	7
IX	tetrahydroisoquinoline	cherylline	8
X	phenanthridone/lignoid	crinasiadine	43
XI	clivimine	clivimine	18
XII	ismine	ismine	9

^a For sources, see ref 22. ^b For structures, see Figures 1 (1–9), 2 (10), 3 (18), and 7 (43).

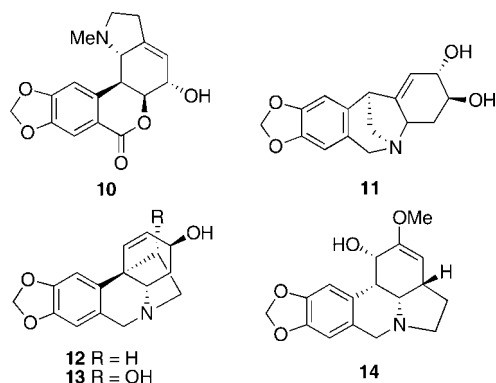


Figure 2. Alkaloids from *A. belladonna* L.: hippeastrine (10), pancracine (11), vittatine (12), 11-hydroxyvittatine (13), and amarbellisine (14).

groups due to reduced toxicity.²⁹ Narwedine (4, Figure 1) potentiates the pharmacological effects of caffeine, carbazole, arecoline, and, to a lesser extent, nicotine, in laboratory animals. In addition, narwedine and vittatine (12, Figure 2) potentiate the analgesic effects of suboptimal doses of morphine.²⁹

Earlier studies also showed that a number of Amaryllidaceae alkaloids cause a transient fall in blood pressure in laboratory animals in high doses. Indeed, narwedine, galanthamine, and *epi*-galanthamine (4, Figure 1; 32 and 33, Figure 6) were reported to produce significant hypotensive effects in mice.^{29,30} Another area of interest has been the acetylcholinesterase inhibitory activity of galanthamine and its ability to amplify the nerve–muscle transfer.³⁰ Extracts of several Amaryllidaceae plants were also found to possess pronounced antibacterial and antifungal activities.³¹

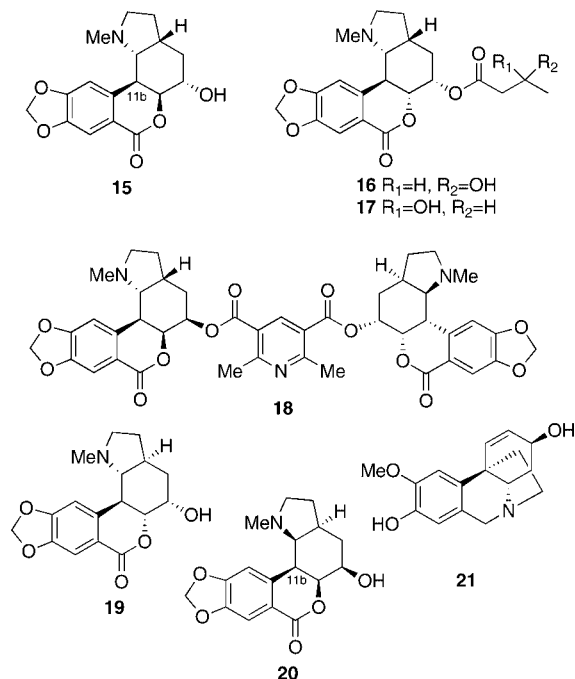


Figure 3. Alkaloids from *Clivia* spp.: clivonine (15), clivatine (16), clivimine (18), nobilisine (19), nobilisitine A (20) and B (17), and (+)-8-demethylmaritidine (21).

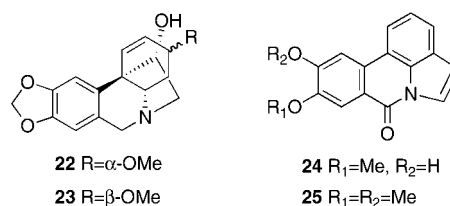


Figure 4. Selected alkaloids from *Crinum* spp.: crinamine (22), hyamine (23), pratorimine (24), and pratosine (25).

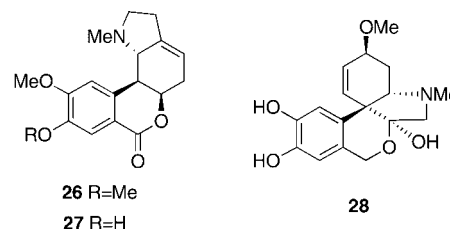


Figure 5. Alkaloids from *N. tazetta* L.: homolycorine (26), its 9-*O*-demethyl derivative (27), and tazettine (28).

Amaryllidaceae alkaloids have repeatedly shown antitumor potential and, among other characteristics, exhibited *in vivo* activity against various human viruses.¹⁸ Several members of the Amaryllidaceae family are toxic and cause symptoms

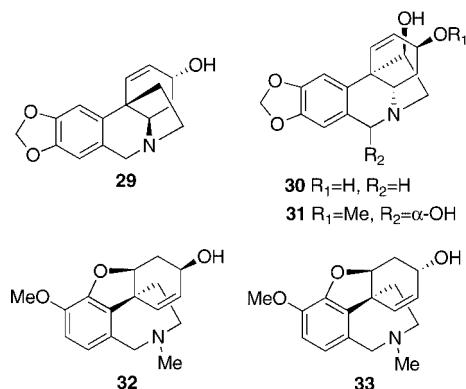


Figure 6. Alkaloids from *Leucojum aestivum*, *N. tazetta* ssp. *tazetta*, and *Pancreatium maritimum* exhibiting antimalarial activity: crinine (**29**), 3-*epi*-hydroxybulbospermine (**30**), 6-hydroxyhaemanthamine (**31**), galanthamine (**32**), and 3-*epi*-galanthamine (**33**).

such as headaches, excessive salivation, nausea, dizziness, heartbeat irregularities, visual disturbance, and dermatitis. Some toxic principles include lycorine and haematim, and the inappropriate use of a number of species can be fatal. Nevertheless, other species are administered orally as medicine to children or eaten in porridge by local people.¹⁸

A number of species of the genus *Amaryllis* have been used in folk medicine, including *Amaryllis belladonna* L. (also named *Hippeastrum equestre*), which is cultivated in Egypt as an ornamental plant.³² Six alkaloids have been isolated from the bulbs of this species, namely, lycorine (**3**, Figure 1), hippeastrine, pancracine, vittatine, 11-hydroxyvittatine, and amarbellisine (**10–14**, Figure 2).³³ The results of antifungal and antibacterial screening showed that amarbellisine, pancracine, vittatine, and 11-hydroxyvittatine have antibacterial activity against Gram-negative *Escherichia coli*, while pancracine also showed activity against *Pseudomonas aeruginosa*. Furthermore, all alkaloids, especially lycorine, amarbellisine, and hippeastrine, showed antifungal activity against *Candida albicans*.³³

Some members of *Ammocharis*, a widely distributed genus, generally inhabit seasonal wet places. *Ammocharis coronica*, a species that contains biochemicals such as alkaloids and triterpenoids in its bulbs, is known to be toxic. Therefore, instead of oral administration, fresh, wet scales are cooked and used as enemas for blood cleansing or applied topically to open wounds or boils.¹⁸

Plants of the *Bophane* genus are known for their large bulbs and production of characteristic alkaloids. *Bophane disticha* (L.f.) Herb. is known as a toxic plant, containing compounds with alleged hallucinogenic potential. However, bulb scales or infusions are used on septic wounds and external sores, as well as for rheumatism and relief of pain. Decoctions are also used for the treatment of headaches, cramps, and internal pains.¹⁸

The *Brunsvigia* genus produces large bulbs containing a number of alkaloids, shown to have significant antimalarial, cytotoxic, and antineoplastic activities. The bulbs are applied as antiseptic dressings on fresh wounds, whereas bulb decoctions are administered for the treatment of coughs, colds, and abdominal, renal, and liver complaints.¹⁸

Clivia species, namely, *Clivia miniata* and *Clivia nobilis*, are cultivated in Egypt as ornamental plants for their beautiful flowers. A root infusion of *C. miniata* (Lindl.) Regel is used to treat snake bites and wounds. In addition, its roots and leaves are taken by South African women during pregnancy and child birth. Aqueous leaf extracts have proven to

augment or induce labor. Bulb decoctions are also used against infertility and urinary complaints.¹⁸ A number of *Clivia* species have been reported to contain Amaryllidaceae alkaloids represented by the 3a,4-dihydro-2H-benzopyrano[3,4-g]indole ring system. This includes clivonine (**15**, Figure 3), isolated from *C. miniata*,³⁴ and clivatine,³⁵ clivimine,³⁶ nobilisine,³⁵ and nobilistine A and B³⁷ (**16**, **18**, **19**, **20** and **17**, Figure 3) isolated from *C. nobilis*. (+)-8-Demethylmaritidine (**21**, Figure 3), a crinine type alkaloid, was also isolated from this species. The antimicrobial activity of the alkaloid extract from *C. nobilis* was tested against Gram-positive (*Staphylococcus aureus*) and Gram-negative (*E. coli* and *P. aeruginosa*) bacterial strains as well as fungi (*C. albicans*). All alkaloids with the exception of clivimine showed antibacterial activity against Gram-positive *S. aureus*. The same compounds, in particular, nobilistine B, exhibited antifungal activity against *C. albicans*.³⁶

Species of *Crinum* genus are large, showy plants with umbels of like flowers. They are found in tropical and subtropical regions throughout the world, and for centuries, they have been used traditionally to cure ailments and diseases.³⁸ One of the earliest recorded uses of *Crinum* is as a powerful emetic. In Asia and America, the bulbs were used to “hasten the ripening of indolent tumors”, while *Crinum zeylanicum* was used in the Moluccas as a strong poison.³⁸ *Crinum asiaticum* is known throughout southeast Asia and Polynesia as an emetic and diaphoretic, while *Crinum bulbispermum* is a general medicinal herb. In southern Sotho, different parts of this plant are used to treat colds and coughs and as an external application or wash for wounds, scrofula, and hemorrhoids. This plant is also used by Zulu, Xhosa, and Sotho people as a gynecological remedy and charm. In Lesotho, it is used as a medicine to increase milk flow, and in India, it is used as a rubefacient in the treatment of rheumatism. *Crinum delagoense* plants are used in Zhulu and Xhosa traditional medicine to treat urinary problems, while in Australia, the aborigines obtain starch from *Crinum flaccidum* to eat as a kind of gruel. *Crinum giganteum* is a Congo leprosy remedy. *Crinum jagus* is used as a veterinary medicinal plant in Cameroon, while in Nigeria, it is used to treat open sores and in an anticonvulsant preparation. In Tanganyika, the fruits and the inner bulb scales of *Crinum kirkii* are used as a purgative, and the outer bulb scales are used as a rat poison. *Crinum macowanii* plants are used by traditional medical practitioners in Zimbabwe to treat backache and also in a number of principal uses, namely, pus disease, blood cleansing, kidney and bladder disease, glandular swelling, fever, infected sores, boils, and acne. *Crinum moorei* bulbs in a decoction are used for swelling and urinary problems, while *Crinum pratense* is used in Indian medicine for intestinal diseases such as diarrhea and dysentery.³⁸ *Crinum latifolium* grows abundantly in the upper Gangetic Plain and is also cultivated as a garden flower. Extracts of different parts of this species are used in popular medicine for a variety of purposes, for example, a rubefacient in rheumatism and piles and for abscess treatment to promote suppuration. Two new pyrrolophenanthridone alkaloids, pratorimine and pratosine (**24** and **25**, Figure 4), were isolated from the bulbs of *Crinum latifolium* and characterized on the basis of comprehensive spectral analysis, chemical transformations, and synthesis.³⁹ The bulbs of *C. jagus* and *Crinum glaucum* are used in traditional medicine in southern Nigeria for memory loss and other mental symptoms associated with aging. Alkaloidal extracts of bulbs from each

species showed inhibition of acetylcholinesterase, an activity exploited therapeutically to raise the depressed levels of acetylcholine in the brain associated with Alzheimer's disease. A number of alkaloids were isolated from this extract, and their activities were quantified. The most active alkaloids isolated were hyamine (**23**, Figure 4) and lycorine (**3**, Figure 1), while other alkaloids were comparatively inactive, such as haemanthamine and crinine (**5**, Figure 1; and **22**, Figure 4). Cholinesterase activity appears to be associated with the presence of two free hydroxyl groups in this structural type of Amaryllidaceae alkaloids.⁴⁰

The use of *Crinum* extends to animals, such as the treatment of weight loss, low milk production, milk loss, or for healthy calves and placenta retention in cattle.³⁸ The reason for the use of *Crinum* species for similar medicinal purposes in different countries is possibly due to their alkaloidal constituents, which in some instances are common to many species. Phytochemical analysis has recently yielded a vast array of compounds, including 150 different alkaloids.³⁹ The structures of the *Crinum* alkaloids, although diverse, are derived from three fundamental nuclei, viz. *N*-(3,4-dioxybenzyl)-4-oxyphenethylamine (e.g., norbelladine, **1**, Figure 1), pyrrolo[*de*]phenanthridine (e.g., lycorine, **3**, Figure 1), and 5,10*b*-ethanophenanthridine (e.g., vittatine or crinine, **12**, Figure 2; and **29**, Figure 6). Various trivial names of *Crinum* alkaloids, their distributions, and biological activities have been reviewed by Ghosal et al.²²

Species belonging to the genus *Cyrtanthus* are among the plants used in South African traditional medicine. *C. mackenziei* is used as a protective charm against storms and evil. Other related species are used to treat diseases like scrofula, chronic coughs, headache, cystitis, and leprosy as well as during pregnancy and child birth. The distribution of the genus *Cyrtanthus*, comprising 51 species in southern Africa, encompasses tropical Africa. In South Africa, *Cyrtanthus* species are mainly distributed in the Western and Eastern Cape province with smaller distribution in Transvaal and KaZulu-Natal.⁴¹

The *Gethyllis* genus comprises 32 species found mainly in southern Africa (Namibia and South Africa). They are widely distributed in the Western and Northern Cape. The genus is endangered, and little is known about its chemistry and biological activities. *Gethyllis* species are used traditionally in South African folk medicine. *G. ciliaris* is used in the Cape as a remedy for colic, flatulence, and indigestion. The fruit pods of many *Gethyllis* species are boiled or administered as an alcohol infusion of brandy for stomach disorders, while flower decoctions are used for toothache.⁴¹ Organic extracts of *Cyrtanthus* species show antibacterial and anti-inflammatory activities, with the most potent obtained from leaves and/or roots of *C. falcatus* and *G. ciliaris*.⁴²

The *Haemanthus* genus produces several alkaloids.²² These perennial plants are used against coughs, dropsy asthma, and as topical antiseptics. Some species have shown RNA antiviral (e.g., Poliovirus) and antineoplastic activities but are also reported for their toxicity, probably due to the presence of alkaloids.¹⁸

N. tazetta L. grows widely in Egypt. It is also cultivated as an ornamental plant and for the production of volatile oil. A number of alkaloids were isolated^{5,23–25} from this species, which are of great interest because of their effective antitumor and antiviral activities, particularly against the choriomeningitis virus.⁴² Wild plants were found to contain

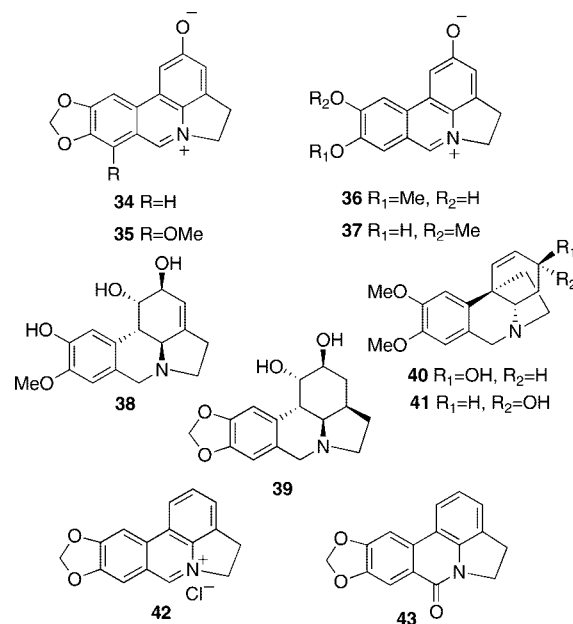


Figure 7. Phenanthridinium betaine alkaloids from *Pancratium* and *Zephyranthes* spp.: ungeremine (**34**), zeflabetaine (**35**), zefbetaine (**36**), *iso*-zefbetaine (**37**), pseudolycorine (**38**), α -dihydrolycorine (**39**), maritidine (**40**), and (+)-*epi*-maritidine (**41**) as well as lycorine oxidation derivatives anhydrolycorinium chloride (**42**) and anhydrolycorine lactam (=crinasiadine, **43**).

lycorine (**3**, Figure 1), homolycorine, 9-*O*-demethylhomolycorine, and tazettine (**26**, **27**, and **28**, Figure 5).^{43,44}

The alkaloids isolated from *N. tazetta* ssp. *tazetta* as well as *Pancratium maritimum* and *Leucojum aestivum* appear to belong to four groups, namely, lycorine, crinine, tazettine, and galanthamine types. Isolated lycorine, haemanthamine (**1** and **5**, Figure 1), tazettine (**28**, Figure 5), crinine, 3-*epi*-hydroxybulbispermine, and 6-hydroxyhaemanthamine (**29–31**, Figure 6) were tested for antimalarial activity against *Plasmodium falciparum*. All four groups of alkaloids exhibited antimalarial activity, albeit at different potencies. 6-Hydroxyhaemanthamine, haemanthamine, and lycorine were found to be the most potent inhibitors of *P. falciparum*, while galanthamine and tazettine were least potent.⁴⁵

The *Pancratium* genus has attracted considerable attention due to the complex structural types of its alkaloids²⁵ and significant therapeutic properties of some of these compounds.⁴⁶ Earlier investigation on the Egyptian *P. maritimum* L. resulted in the isolation of lycorine, tazettine, pancracine, lycorenine, galanthamine, sickenbergine, homolycorine, haemanthidine, hippadine, demethylhomolycorine, and trisphe-ridirine⁴⁷ and then haemanthamine, pseudolycorine, and 11-hydroxyvittatine.^{48,49} Successively from the same plant were isolated two 2-oxyphenanthridinium betaine type alkaloids, ungeremine and zefbetaine⁵⁰ (**34** and **36**, Figure 7), known for their very interesting biological activities (i.e., cytotoxic, antibiotic,^{51–53} and plant growth-regulatory⁵¹ activities). The corrected structure of zefbetaine, deduced from a more accurate spectroscopic and chemical analysis, appeared in contrast with that reported in its first isolation from *Zephyranthes flava*.⁵¹ In fact, this structure determination was also supported by the partial synthesis of zefbetaine from pseudolycorine (**38**, Figure 7), an alkaloid isolated from *P. maritimum* L. together with α -dihydrolycorine (**39**, Figure 7),⁴⁸ and by comparison with its unnatural isomer *iso*-zefbetaine (**37**, Figure 7).^{50,54} Ungeremine is also found in *Ungernia minor*,⁵⁵ *Crinum americanum*,⁵⁶ *C. asiaticum*,⁵⁷

and *Z. flava*.⁵¹ Interestingly, this natural product can be obtained by microbial transformation of lycorine performed by a *Pseudomonas* sp. isolated from the rizosphere of *S. lutea*. Together with ungeremine and zefbetaine, two other phenanthridinium derivatives were isolated and characterized as anhydrolycorinium chloride (**42**, Figure 7) and the dihydro derivative of ungeremine. The evidence obtained demonstrates that the transformation occurs through oxidative aromatization of lycorine.⁵³ Many of these microbially aromatized compounds, including lactam **43** (criasiadine, Figure 7), showed good antibiotic activity when tested against *Corynebacterium fascians*.⁵³

In the genus *Zephyranthus*, *Z. parulla* Killip appears in the history of Peru (by Padre Cobo, 1653) for treating tumors, and *Z. Rosea* (Spreng.) Lindl. has found use in China for treating breast cancer.¹ The leaves of *Z. candida* (Lindl.) Herb. have been employed in Africa as a treatment for diabetes mellitus.⁵⁸ The bulbs from a 1964 Hong Kong collection of these plants produced an extract, which showed good anticancer activity (cell line from human epidermoid carcinoma of nasopharynx, KB system) in the U.S. National Cancer Institute's (NCI) research programs. *Z. flava* Roem & Schult is a deciduous herb native to America, which is naturalized in India. The plant grows abundantly in the upper Gangetic plains and bears yellow flowers during the rainy season (July–August). The extract of its bulbs is used in the indigenous system of medicine for a variety of therapeutic purposes, for example, in the treatment of diabetes, for ear and chest ailments, and against viral infections. The bulbs contain a complex mixture of alkaloids and non-nitrogenous aromatic compounds.⁵⁹ Investigations carried out on the fresh mature fruits allowed the identification of the following compounds: eight tertiary alkaloids, crinamine, haemanthamine, lycorine, maritidine, methylpseudolycorine, pretazettine, haemanthidine, and pseudolycorine; two lactam alkaloids, narciclasine and pratorimine; three glucosyloxy alkaloids, kalbreclasine, lycorine-1-*O*- β -D-glucoside, and pseudolycorine-1-*O*- β -D-glucoside; and four phenanthridinium betaine alkaloids, criasbetaine, ungeremine, zefbetaine, and zeflabetaine (**35**, Figure 7).⁵¹ *Z. rosae* similarly grows in the upper Gangetic plain as well as the Sikkim region of the Eastern Himalayas up to 1000 m, and it is also grown in gardens as an ornamental flowering plant and for medicinal purposes. Extracts of flowers and bulbs are used for a variety of therapeutic purposes, which can be described in modern terms as immunomodulators. From the extract of fresh bulb were isolated four alkaloids: crinamine (**22**, Figure 4), haemanthamine (**5**, Figure 1), maritidine, and (+)-*epi*-maritidine (**40** and **41**, Figure 7).⁶⁰

2.2. Future Perspectives

Only 15% of the world's known plant resources have been screened for their therapeutic values.⁶¹ Natural products provide mankind with more environmentally friendly alternatives to commercially produced medicines. Bulbous plants, although studied less intensively than herbs and trees for medicinal potential, have proven to contain a range of unique biologically active compounds. Valuable uses include their analgesic, anticancer, antimutagenic, immunostimulatory, anti-infective, antimalarial, cardiovascular, and respiratory system effects.^{38,62} Traditional uses of some bulbous species, mainly belonging to the Amaryllidaceae and Hyacinthaceae, could provide useful leads in novel pharmaceutical developments. Because the biochemical content and pharmacological

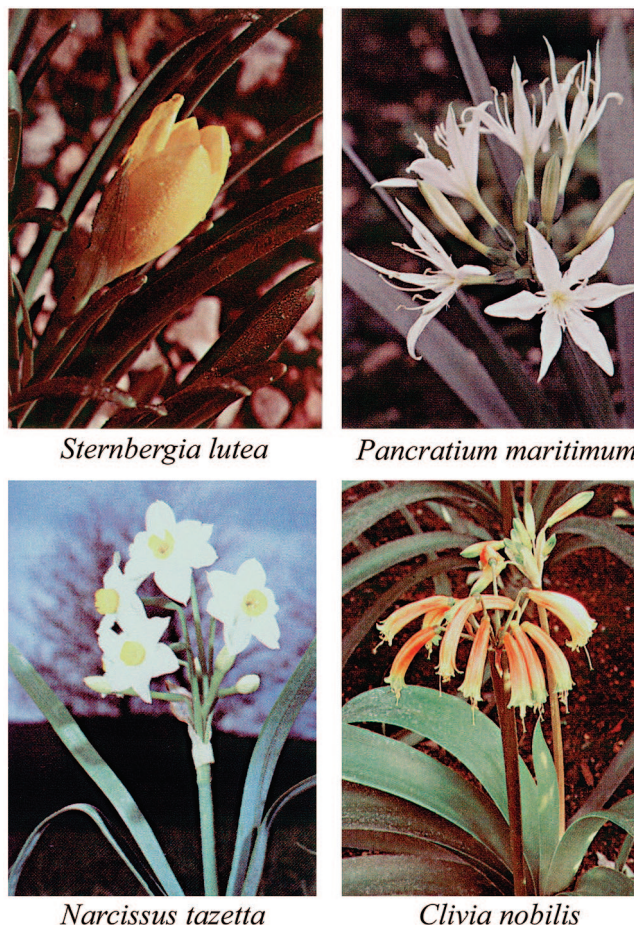


Figure 8. Representative amaryllidaceae plants used in traditional medicine. Reprinted with permission from ref 28. Copyright 2000 Springer Science and Business Media.

action of many of these species still remain poorly understood, future research should be invaluable in this respect. Some of the medicinally important Amaryllidaceae plants discussed in section 2.1 are shown in Figure 8.

2.3. Narciclasine and Its Naturally Occurring Analogues: Plant Sources and Isolation Methods

Narciclasine (1,3,4,5-tetrahydro-2,3,4,7-tetrahydroxy[1,3]dioxolo[4,5-*j*]phenanthridin-6(2*H*)-one, **44**, Figure 9) is not a basic metabolite, since the nitrogen is amidic in character, but its structure is closely related to the Amaryllidaceae lycorine type alkaloids. Narciclasine, isolated for the first time from different varieties of *Narcissus* bulbs⁶³ in a systematic program aimed at the detection of antigrowth factors in plants, showed potent antimetabolic activity.⁶⁴ The structure of narciclasine was then revised by a chemical study⁶⁵ and unambiguously assigned, including the absolute stereochemistry by an X-ray analysis of the corresponding tetraacetate.⁶⁶ Narciclasine is identical to lycoricidinol, independently isolated from *Lycoris radiata*.⁶⁷ From the bulbs of the same *Narcissus* species, a closely related metabolite was also isolated and named narciprimine (**48**, Figure 9).⁶³ The early structure of narciprimine was successively revised by a chemical study⁶⁵ and found to be identical to arolycoridinol obtained by acid treatment of lycoricidinol.^{63,67} Later, narciprimine and narciclasine were also isolated together from *Zephyranthes tubispatha*⁶⁸ and *Lycoris sanguinea*.⁶⁹

The promising activity of narciclasine has stimulated much effort toward the isolation and chemical characterization of

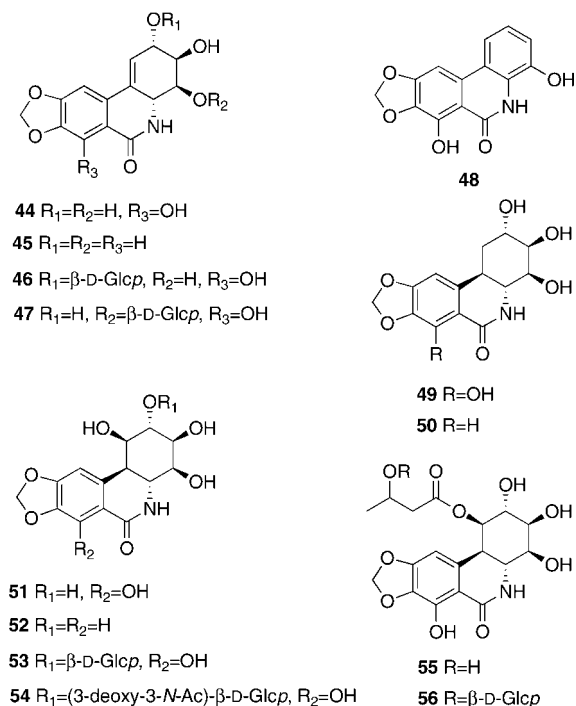


Figure 9. Narciclasine (**44**), pancratistatin (**51**), and their corresponding naturally occurring analogues (**45**–**50** and **52**–**56**).

its naturally occurring analogues from different Amaryllidaceae species. 7-Deoxynarciclasine (**45**, Figure 9, often referred to as lycoricidine in the literature) was isolated from *L. radiata*⁶⁷ and *L. sanguinea*⁶⁹ as well as *Pancreatum littorale*⁷⁰ (later reidentified as *Hymenocallis littoralis*, vide infra) and *Haemanthus kalbreyeri*.⁷¹ The last two species were also found to contain narciclasine and a new phenanthridone pancratistatin (**51**, Figure 9).^{70–73} *Haemanthus kalbreyeri* also produces 7-deoxypancratistatin and 2-*O*- β -D-glucoside of pancratistatin (named pancratistide) (**52** and **53**, Figure 9).⁷¹ Two other congeners of narciclasine, *trans*-dihydrnarciclasine and its 7-deoxy derivative (**49** and **50**, Figure 9), were isolated correspondingly from *Z. candida*⁷⁴ and *Hymenocallis littoralis*, *H. caribea*, and *H. latifolia*.⁷² Thus, it appears that all three subtypes of these phenanthridones, namely, narciclasine, pancratistatin, and *trans*-dihydrnarciclasine, occur in both 7-hydroxy and 7-deoxy forms. Interestingly, narciclasine, pancratistatin, and ungeremine were also found in lubber grasshopper *Brachystola magna*. This is the only isolation of isocarbostryl and phenanthridinium alkaloids from an insect.⁷⁵

Finally, a number of glycosyl derivatives of narciclasine and pancratistatin were isolated from different Amaryllidaceae species. As mentioned above, pancratistide is isolated from *H. kalbreyeri*,⁷¹ and kalbreclasine and 4-*O*- β -D-glucosyl-narciclasine (**46** and **47**, Figure 9) are isolated, respectively, from *Z. flava*,⁵¹ *H. kalbreyeri*,⁷⁶ and *P. maritimum*.⁷⁷ In addition, three others derivatives of pancratistatin, 1-*O*-(3-deoxy-3-*N*-Ac)- β -D-glucopyranosyl-pancratistatin (**54**, Figure 9), named telastaside, 1-*O*-(3-hydroxybutyryl)-pancratistatin, and 1-*O*-(3-*O*- β -D-glucopyranosylbutyryl)-pancratistatin (**55** and **56**, Figure 9), were isolated from the smokey-gray moth *Polytela gloriosa* (**54**)⁷⁸ and *Z. carinata* (**55** and **56**).⁷⁹

Narciclasine and its naturally occurring analogues were isolated from different commercially available, wild, and cultivated in botanical gardens Amaryllidaceae species as summarized in Table 2.

The processes used for the extraction and purification of these isocarbostryl plant constituents differ in the methodology and yields of products, which are frequently obtained in crystalline form (Table 2). The major stages of these processes include extraction of the fresh plant material with a single or a mixture of solvents, purification of the crude organic extract, or the extract liberated from bases (including essentially alkaloids) by chromatography, followed by a final crystallization of the isocarbostryl compounds. The established processes could be summarized as follows.

2.3.1. Method A

The fresh ground bulbs of the analyzed Amaryllidaceae plant are extracted with 95% ethanol and left to macerate for 1 day. The ethanol extract is filtered, concentrated, subjected to a complex process of clearing, and then extracted with *n*-butanol. The *n*-butanol extract is fractionated by a silica gel column, and crude phenanthridones so obtained are crystallized from a suitable solvent.⁶³

2.3.2. Method B

The fresh ground bulbs of the analyzed Amaryllidaceae plant are extracted with methanol for a longer period of time (3 days). The crude concentrated extract is subjected to successive extractions with ethyl acetate and finally fractionated by a silica gel column. The crude phenanthridones are crystallized from a suitable solvent mixture.⁶⁷ Alternatively, the residual aqueous phase of the initial methanol extract is extracted in succession with petrol, ethyl acetate, and *n*-butanol. The residue of *n*-butanol extract is dissolved in a small volume of methanol-dioxane and filtered, and the filtrate is kept at room temperature until a brown solid separates. The latter is dissolved in methanol and fractionated by a silica gel column to yield crude phenanthridones, which are crystallized from a suitable solvent.⁷⁶

2.3.3. Method C

The fresh plant material is macerated in aqueous methanol in a high-speed blender and filtered, and the filtrate is evaporated under reduced pressure to give a viscous brown slurry. This is triturated with hot petrol to remove fatty acid materials and weakly polar alkaloids. The petrol-insoluble fraction is treated with aqueous acetic acid, and a brown solid that separates is collected. The cleared acid solution is extracted with ethyl ether, then basified, and extracted in succession with chloroform, ethyl acetate, and *n*-butanol. These organic fractions are independently worked on to purify alkaloids, phenanthridones, and phenanthridinium and glycosyl alkaloids, respectively.⁵¹

2.3.4. Method D

This method involves an initial extraction of ground plant material (essentially bulbs) with a methanol-methylene chloride (1:1 v/v) for a long (15–20 days) period of time. The methylene chloride phase is separated by the addition of water. The aqueous phase is adjusted by addition of further amounts of methanol and methylene chloride, and the bulbs are re-extracted for the same amount of time. Decantation and addition of water separate the methylene chloride phase. The aqueous phase is concentrated and centrifuged, and the resulting clear solution is extracted with *n*-butanol. The *n*-butanol extract is concentrated, and its aliquot is dissolved

Table 2. Occurrence of Narciclasine and Its Natural Congeners in the Amaryllidaceae^a

compound (structure)	Amaryllidaceae	content (mg/kg) ^b	ref	
narciclasine (lycoricidinol, 44)	<i>Brachystola magna</i>	1.5	75	
	<i>Galanthus nivalis</i> L.	10	15	
	<i>G. elwesii</i> Hook	25	15	
	<i>Narcissus</i> spp.	30–200	15	
	<i>Haemanthus puniceus</i> L.	<5	15	
	<i>H. kalbreyeri</i>	4.4 ^c	71	
	<i>Hymenocallis littoralis</i>	ND	63	
	<i>H. speciosa</i>	2.0	82	
	<i>H. expansa</i>	3.0	82	
	<i>Leucojum aestivum</i> L.	30	15	
	<i>L. Vernum</i> L.	50	15	
	<i>Lycoris radiata</i> Herb.	41.6	67	
	<i>L. sanguinea</i>	ND	69	
	<i>N. tazetta</i> L.	65	81	
	<i>Pancratium littorale</i>	ND	72	
	<i>P. maritimum</i> L.	50	15	
	<i>Sprekelia formosissima</i> (L.) Herb	>5	15	
	<i>Sternbergia lutea</i> (L.) Ker-Gawl	<5	15	
	<i>Vallota speciosa</i> L'Her	<5	15	
	<i>Zephyranthes flava</i>	135.0 ^d	51	
	<i>Z. tubispatha</i>	ND	68	
	narciprimine (48)	<i>L. sanguinea</i>	ND	69
		<i>Narcissus</i> spp.	3–5	63
<i>Z. tubispatha</i>		ND	68	
7-deoxynarciclasine (lycoricidine, 45)	<i>Lycoris radiata</i> Herb.	31.9	67	
	<i>L. sanguinea</i>	ND	69	
	<i>H. littoralis</i>	222.2	70, 72	
<i>trans</i> -dihydronarciclasine (49)	<i>H. kalbreyeri</i>	1.7 ^c	71	
	<i>Z. candida</i>	ND	74	
	7-deoxy- <i>trans</i> -dihydronarciclasine (50)	<i>H. littoralis</i>	33.0	72
		pancratistatin (51)	<i>B. magna</i>	2.5
	<i>H. kalbreyeri</i>		15.8 ^c	71
	<i>H. littoralis</i>	144.4	70, 72, 73	
	<i>H. expansa</i>	4.0	82	
	<i>H. pedalis</i>	28.9	82	
	<i>H. sonorensis</i>	3.0	82	
	<i>H. speciosa</i>	3.0	82	
	<i>H. variegata</i>	6.0	82	
<i>P. maritimum</i>	6.0	82		
7-deoxypancratistatin (52)	<i>Z. grandiflora</i>	19.1	80	
	<i>Z. carinata</i>	7.5 ^e	79	
	kalbreclasine (46)	<i>H. kalbreyeri</i>	2.7 ^c	71
<i>H. kalbreyeri</i>		3.1 ^c	76	
4- <i>O</i> -β-D-glucopyranosylnarciclasine (47)	<i>Z. flava</i>	165.0 ^d	51	
	<i>P. maritimum</i>	3.3	77	
pancratiside (53)	<i>H. kalbreyeri</i>	19.6 ^c	71	
telastaside (54)	<i>Polytela gloriosa</i>	400	78	
1- <i>O</i> -(3-hydroxybutyryl)pancratistatin (55)	<i>Z. carinata</i>	23.7 ^e	78	
1- <i>O</i> -(3- <i>O</i> -β-D-glucopyranosylbutyryl) pancratistatin (56)	<i>Z. carinata</i>	43.8 ^e	78	

^a ND, not determined. ^b Fresh bulbs or insects (*B. magna* and *P. gloriosa*). ^c Fresh roots. ^d Fresh seeds. ^e Air-dried bulbs.

in methanol. The addition of acetone allows frequently insoluble material to separate. The treatment of this precipitate with methanol causes pancratistatin to separate.^{70,72,80}

2.3.5. Method E

Such an alternative method utilizes mucilage secreted from bulbs. The plant bulbs are chopped in small pieces and immersed in distilled water for 30 h to allow the mucilage to leach out and then washed four times. The mucilage solutions are combined and lyophilized, and the residual powder is extracted with 70% ethanol using a Soxhlet. The extract is concentrated and extracted with ethyl acetate, and the corresponding crude extract is fractionated by a silica gel column yielding a crystalline isocarbostryl constituent.⁸¹

Method A was applied to extract narciclasine from 32 species and varieties of the *Narcissus* genus, resulting in the isolation of the pure phenanthridone in the range of 30–200

mg/kg.¹⁵ The same method was used to extract narciclasine from other Amaryllidaceae genera (Table 2),¹⁵ but a more simplified procedure was used when the extraction was carried out on daffodils particularly rich in narciclasine, avoiding essentially the column chromatographic fractionation.⁶³ As observed for other Amaryllidaceae alkaloids (e.g., the alkaloids extracted from *A. belladonna* L.),³³ the content may vary during the year at the different stages of plant growth: In *N. incomparabilis* Mill var. *helios*, 200 mg/kg of narciclasine was found in March at the flowering stage, but only 100–120 mg/kg was found in November.¹⁵ The same method was used to extract and purify in very low yield narciprimine from *Narcissus* spp. (Table 2).⁶³

Method B was used to extract narciclasine and 7-deoxy-narciclasine from *L. radiata* Herb.; the yields for each isocarbostryl metabolite, crystallized respectively from water and methanol,⁶⁷ are given in Table 2. Similarly, narciclasine,

kalbreclasine, and 7-deoxypancratistatin were isolated from *H. kalbreyeri*,^{71,76} applying a minor modification of this method.

Method C was used to purify narciclasine and kalbreclasine from *Z. flava* seeds. In this case, the residue obtained from the ethyl acetate extraction was treated as in method B.⁵¹

Method D was used to purify narciclasine and some of its congeners, namely, pancratistatin, 7-deoxynarciclasine, and 7-deoxy-*trans*-dihydronarciclasine from *Z. grandiflora*,⁸⁰ *P. maritima*,⁸² and some *Hymenocallis* spp., which represent a broad geographical distribution, such as *H. littoralis* (Hawaii), *H. caribea* and *H. latifolia* (Singapore),⁷² *H. speciosa* and *H. variegated* (Singapore), *H. pedalis* (Seychelles), *H. expansa* (Bermuda), and *H. sonoranesis* (Mexico).⁸² A similar procedure was also used to isolate narciclasine, pancratistatin, and ungeremine from the insect *Brachystola magna*.⁷⁵ This method of extraction followed by purification of the *n*-butanol extract by a silica gel preparative TLC was used to isolate telastaside (**54**) from *P. gloriosa* along with several Amaryllidaceae alkaloids belonging to the subgroups of pyrrolo- and 5,10b-ethano-[*de*]phenanthridine (Table 1). The larva of this insect is a specialized herbivore, which uses Amaryllidaceae plants, avoided by other insects, as an ecological niche.⁷⁸ Telastaside represents the first isolation of an isocarbostyryl derivative from an insect. Improved laboratory and pilot plant scale techniques have also been developed for the isolation of pancratistatin from the difficult-to-separate mixture of narciclasine and its 7-deoxy derivative occurring in *H. littoralis*. This method eliminates the use of *n*-butanol extraction and Sephadex LH-20 chromatography steps that are very effective for laboratory scale isolation of pancratistatin but costly on a large scale. The method proved efficient and rapid.⁸³ Finally, a scale-up method for the isolation of phenanthridones (pancratistatin and related isocarbostyryls) from *H. littoralis* was developed. The bulbs were subdivided and placed in six big steel containers (120 L). Technical grade methanol was added to each vessel. After extraction for 20–48 days, the methanol phase was removed and evaporated to give a water-concentrated solution. A second extraction was carried out with the same solvent for 16–24 days. The concentrated aqueous phases were combined and extracted five times with methylene chloride. The organic phase was concentrated to dryness, and the residue was partitioned with ethyl acetate and then with *n*-butanol. These fractions were evaporated to dryness and dissolved in methanol, and the addition of acetone allowed lycorine to separate. The soluble fraction was concentrated and dissolved in methanol, filtered, and fractionated by gel permeation using a Sephadex LH-20 column chromatography eluted with methanol. Some fractions contained the above-mentioned phenanthridones pancratistatin, narciclasine, and its 7-deoxy and 7-deoxy-*trans*-dihydro derivatives.⁸⁴

Method E was applied to isolate narciclasine from the bulbs of *N. tazetta* L.⁸¹ The phenanthridone was obtained in the same yield when bulbs of the same plant were extracted with 95% ethanol according to the classical method A.⁶³

2.3.6. Method F

An alternative improved method F with respect to all of the above-described procedures was developed by Evidente. It avoids the use of organic solvents (ethanol, methanol, or methanol combined with methylene chloride). The method is based on the acidic properties of the amidic nitrogen of

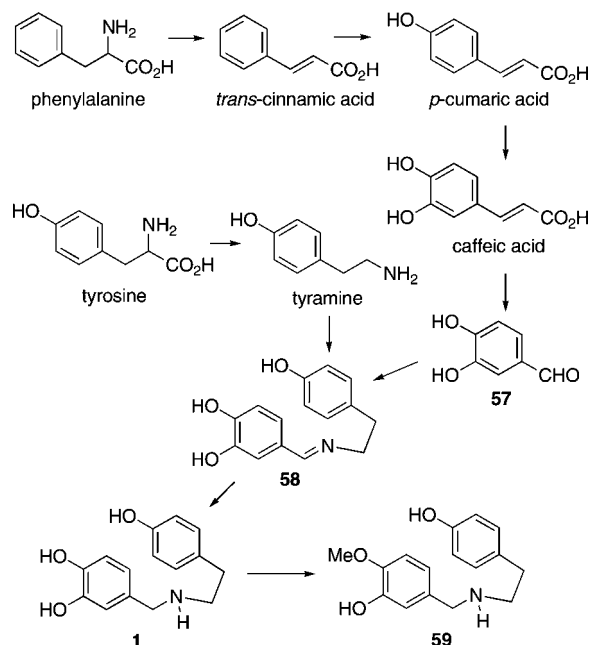


Figure 10. Biosynthesis of *O*-methylnorbelladine (**59**).

narciclasine. The air-dried and milled plant material is extracted with an aqueous solution of sodium hydroxide. The mixture is left to macerate overnight at room temperature, and then, it is filtered and centrifuged. The aqueous solution is acidified at pH 2 and extracted three times with ethyl acetate. The organic extracts are combined, dried, and evaporated under reduced pressure, and the residue is purified by silica gel column. Narciclasine is crystallized from acetic acid.⁸⁵ When this procedure was applied to *S. lutea* Ker-Gawl bulbs, narciclasine was obtained in higher yield (20 mg/kg) than that (<5 mg/kg) obtained using the classical ethanol extraction method A.^{63,15}

3. Biosynthetic Studies

3.1. Biosynthesis of Amaryllidaceae Alkaloids

Several studies on the biosynthesis of Amaryllidaceae alkaloids belonging to different ring type subgroups have been carried out and reviewed.⁸⁶ *O*-Methylnorbelladine (**59**, Figure 10), which plays a central role in the biosynthesis of these alkaloids, is formed from phenylalanine and tyrosine by the route illustrated in Figure 10. Phenylalanine is a precursor of protocatechuic aldehyde (**57**), which condenses with tyramine, derived from tyrosine, to yield Schiff base **58**. Reduction of the latter affords norbelladine (**1**), which is methylated to *O*-methylnorbelladine (**59**).

Para-ortho oxidative coupling of *O*-methylnorbelladine (**59**) yields the transient intermediate **60** (Figure 11). Nucleophilic attack of the amino group on the dienone produces norpluvine (**61**). This, in turn, by allylic oxidation and formation of the methylenedioxy group from the *O*-methyl group of **59**,⁸⁷ yields lycorine (**3**). This mechanism is supported by the studies involving appropriately labeled precursors.^{88–90} Lycorine is the precursor of hippastrine (**10**), and the plausible intermediates are **62** and **63** (Figure 11).

Para-para coupling of *O*-methylnorbelladine leads to dienone intermediate **64** (Figure 12), which by intramolecular Michael addition leads to noroxomaritidine (**65**).⁹⁰ The latter, by a series of successive steps, including the conversion of

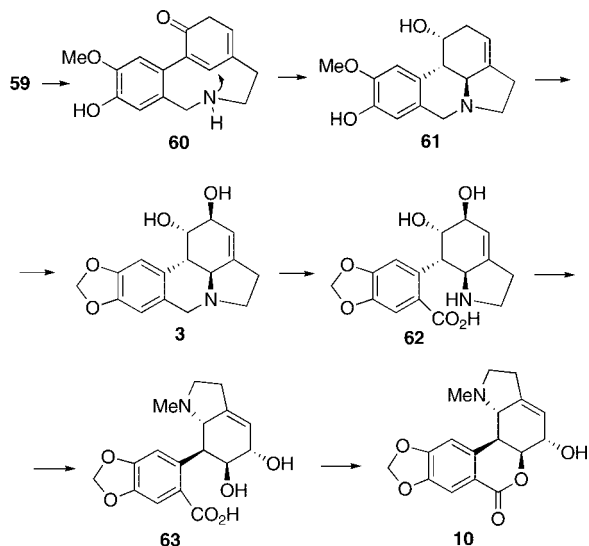


Figure 11. Biosynthesis of Amaryllidaceae alkaloids by *para-ortho* oxidative coupling of *O*-methylnorbelladine.

the *O*-methyl ether of **59**⁸⁷ into the methylenedioxy group, leads to alkaloids such as buphanisine (**66**). Further oxidation yields haemanthamine (**5**),^{91–94} which has been shown to be a precursor of haemanthidine (**67**) and tazettine (**28**). The conversion of intermediate **68** to **69** involves an intramolecular hydride transfer.⁹⁵

The nonheterocyclic alkaloid ismine (**9**), which possesses a C-13 skeleton similarly to narciclasine, is present in trace amounts in a number of Amaryllidaceae plants. Feeding experiments showed biological derivation of ismine from **59** by *para-para* coupling, followed by a late elimination of the “ethano” bridge from the C-15 crinane skeleton. Further evidence was obtained by incorporation in *Sprekelia formosissima* of triply labeled [6,10-³H₂; 12-¹⁴C]noroxomaritidine (**65**) into **9** as well as **5** and **67**.⁹⁶ In addition, a possible link between the biosynthesis of haemanthamine and haemanthidine and that of ismine was demonstrated. Thus, using a doubly labeled [6*R*,10-³H₂]noroxomaritidine as a precursor, evidence of the stereospecificity of the benzylic oxidation in the biosynthesis of **9** was established, in that a pro-*R*-hydrogen from C-6 of noroxomaritidine is removed, which was also observed in the conversion of **5** into **67**.⁹⁶

The inverse *ortho-para* oxidative coupling of *O*-methylnorbelladine represents a third biosynthetic pathway, and it leads to narwedine (**4**) and galanthamine (**32**) as illustrated in Figure 13. In this case, the *N*-methylation to the intermediate **70** is apparently essential prior to oxidative coupling.^{92,97–99}

3.2. Biosynthesis of Narciclasine

Several studies have been carried out to determine the biosynthesis pathway, which leads from *O*-methylnorbelladine (**59**) to narciclasine (**44**), using different and specifically labeled precursors.²⁵

As discussed above, the biosynthesis of several Amaryllidaceae alkaloids involves different phenolic couplings, which generate, through independent pathways, alkaloids that belong to different subgroups, such as lycorine (**3**) and haemanthamine (**5**). Because alkaloids from both of these subgroups are often isolated from the same plants as narciclasine, the latter might arise by either *para-ortho* (Figure 11) or *para-para* (Figure 12) pathways with a late degradation of the C-15 skeleton (Figure 14).

The first experiment involved the study of tritium incorporation from a suitable *ortho*-labeled [3,5'-³H₂; ¹⁴C-*O*-methyl]-**59** in daffodils into lycorine, haemanthamine, and narciclasine, followed by chemical investigations of specific sites of incorporation in the radioactive narciclasine and haemanthamine. Lycorine, haemanthamine, and narciclasine showed a 50, 100, and 75% tritium label retention, respectively. The 75% retention observed in narciclasine as compared with the expected 50% retention shown by lycorine pointed to the derivation of this isocarbostryl from **59** following the *para-para* coupling pathway.²⁵ Indeed, narciclasine, upon aromatization to narciprimine (**48**) in acidified heavy water, lost ca. 30% of the ³H activity without picking up of deuterium from the solvent. Because in this conversion the hydrogen atoms at positions C4 and C4a of narciclasine are removed, the tritium, which is lost, should be located at one or both of the above-mentioned positions. However, because of the lack of radioactivity of narciclastic aldehyde (**72**, Figure 14), which retains the C4a hydrogen, the removed ³H atom was assigned to position C4.²⁵ The results support the derivation of lactam **44** from **59** along the *para-para* oxidative coupling pathway.

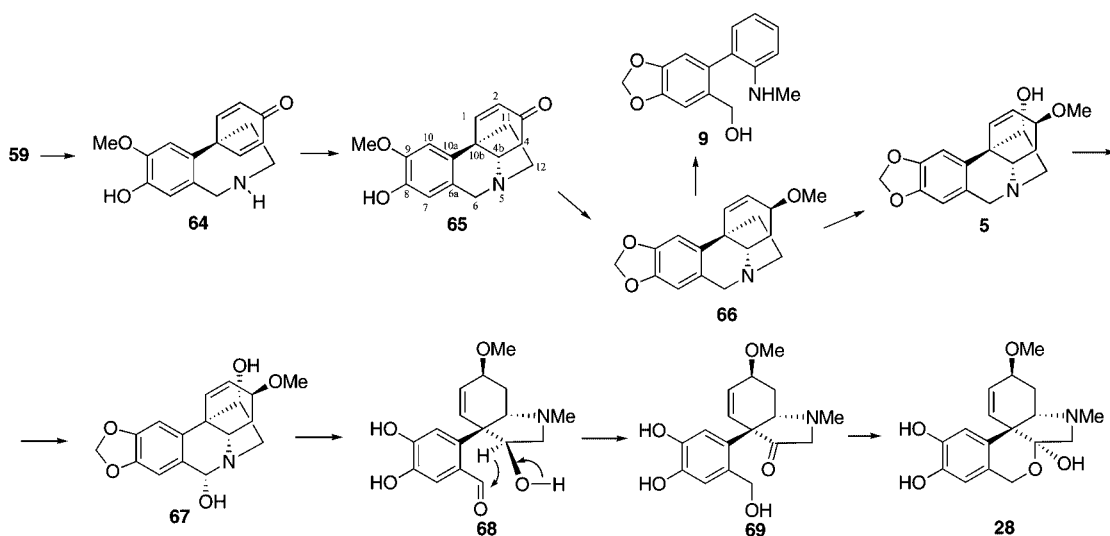


Figure 12. Biosynthesis of Amaryllidaceae alkaloids by *para-para* oxidative coupling of *O*-methylnorbelladine.

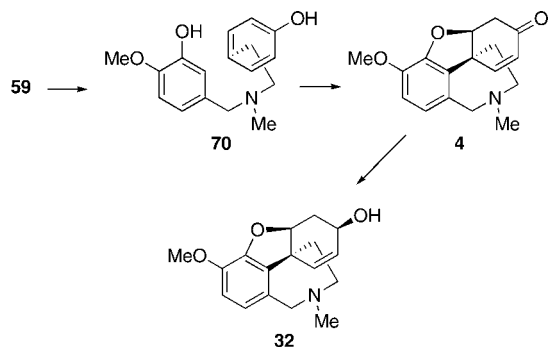


Figure 13. Biosynthesis of Amaryllidaceae alkaloids by a third mode of oxidative coupling of *O*-methylnorbelladine.

Further evidence was obtained by incorporation of a *meta*-labeled [2',6'-³H₂; ¹⁴C-*O*-methyl]-**59** in narciclasine, haemanthamine, and norpluvine, followed by similar chemical investigations of the specific incorporation sites in radioactive narciclasine. Narciclasine (**44**) and haemanthamine (**5**) incorporated the precursor without significant ³H loss, while norpluvine (**61**) showed ca. 15% tritium loss. The ³H labels were assigned to positions C1 and C4a of narciclasine because narciclastic aldehyde (**72**) retained all of the ³H activity and narciprimine (**48**) formed with 50% ³H loss. The latter lost all of the ³H activity after the hydrogens *ortho* and *para* to the phenolic group of the C ring were substituted for deuterium by base-catalyzed exchange of a suitable derivative.²⁵

These tracer experiments confirmed that narciclasine is biosynthesized from *O*-methylnorbelladine by *para*–*para* coupling followed by a late elimination of two carbon atoms.¹⁰⁰ Further work has been carried out to define the nature of the intermediates between **59** and narciclasine. It involved the use of suitably labeled intermediates, such as racemic [1,4b-³H₂]noroxomaritidine (**65**) and racemic [3-³H₃]vittatine (**12**). The radioactive labels of both noroxomaritidine and vittatine were incorporated into narciclasine, which was subjected to chemical degradation to investigate the specific sites of radioactivity incorporation. The labeling pattern of radioactive narciclasine was determined by degradation to narciprimine (**48**), which, after methylation, was brominated at positions C1 and C2 as well as by oxidation of narciclasine to narciclastic aldehyde (**72**). The obtained relative ³H-molar activities of the degradation series point to the following conclusions: (i) the conversion of **59** into narciclasine proceeds through its phenol-coupled product

65 and through intermediates bearing a pseudoaxial hydroxyl group at the position C3 of the crinane skeleton; (ii) the hydrogen removal from position C3 during the biosynthesis does not occur; (iii) enzymes that are able to form a methylenedioxy group of the crinane skeleton irrespectively of the oxidation level at C-3 are involved.¹⁰¹

Additional experiments showed tritium incorporation from suitably labeled [2,4-³H₂]11-hydroxyvittatine (**13**) in *P. maritimum* into narciclasine (5%) and haemanthamine (3%). Radioactive **13** had been previously obtained from *P. maritimum* in several feeding experiments with [3',5'-³H₂]-**59**. The labeling pattern of the radioactivity of narciclasine was determined by chemical degradation of narciclasine into narciprimine (**48**), which in confirmation of the previous findings, retained about 70% of the starting tritium activity. The results obtained establish the intermediacy of **13** in the biosynthesis of narciclasine and confirm the hydroxylation β to the nitrogen in the degradation of the alkaloidal skeleton.¹⁰² These findings also provide experimental evidence for the relevant steps in the biosynthetic route leading from **59** to narciclasine involving the loss of the “ethano” bridge from the intermediates possessing the crinane skeleton. 11-Hydroxyvittatine (**13**) could be the last intermediate, possessing the “ethano” bridge, and it might undergo the C–C bond cleavage in a mechanistically acceptable manner. Alkaloid **13** might undergo a *retro*-Prins reaction to give an intermediate like **71**, which in a few oxidative steps could give narciclasine.¹⁰³ A similar mechanism should occur in the biosynthesis of the alkaloid ismine (**9**), which possesses a C-13 skeleton as does narciclasine and, as mentioned above, is derived from oxocrinine through elimination of the “ethano” bridge.

3.3. Biosynthesis of Narciclasine's Congeners

There are still now no reports in the literature on the biosynthesis of the narciclasine and pancratistatin analogues. Some indirect results on the biosynthesis of pancratistatin were obtained by developing a biotechnological approach, which involved a large culture tissue cloning operation of *H. littoralis* bulbs. These “seedlings” replicas were transplanted to potting soil under greenhouse conditions and the final planting in native soil, exposing the plant to light and temperature conditions typical of arid Tempe, Arizona.¹⁰⁴ The plant tissue culture → greenhouse → field production sequence was successively utilized to increase an original 1.5 kg of wild *H. littoralis* bulbs to some 60000 bulbs at

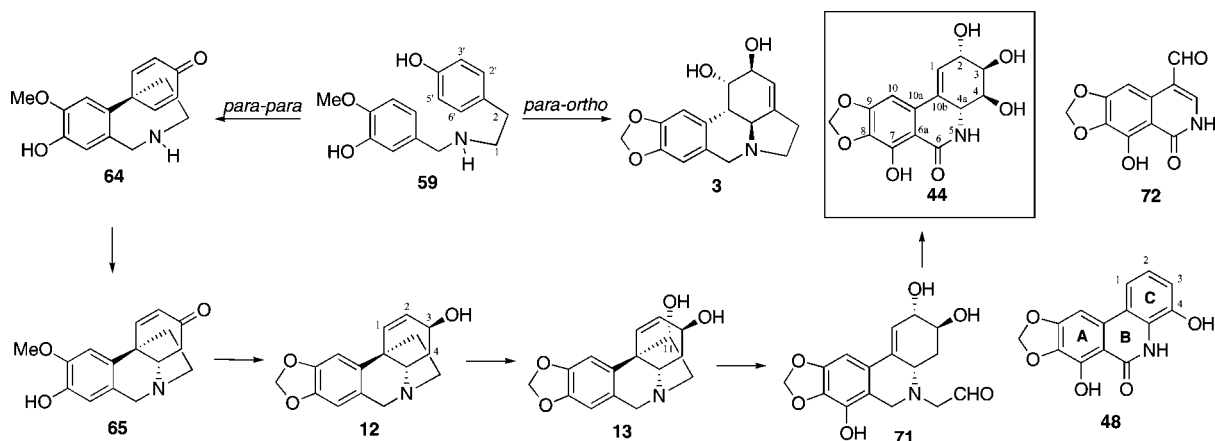
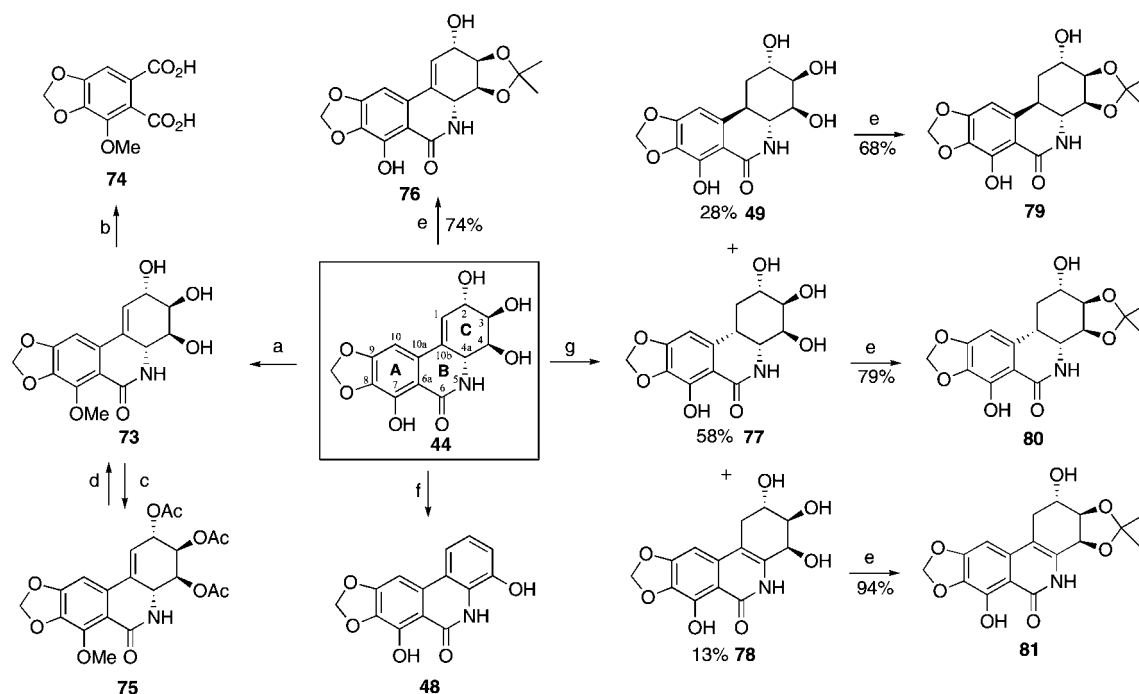


Figure 14. Biosynthesis of narciclasine (**44**).

Scheme 1^a

^a Reagents and conditions: (a) CH_2N_2 (excess), EtOH, 48 h. (b) KMnO_4 , H_2O , 0 °C, 48 h. (c) Ac_2O , py, 48 h, room temperature. (d) $\text{Ba}(\text{OH})_2$, MeOH, reflux. (e) Acetone, $\text{HC}(\text{OEt})_3$, TsOH, reflux, 15 h. (f) Concentrated HCl. (g) H_2 , Pd/CaCO₃, EtOH, 3 h.

present. In the Sonoran Desert (central Arizona), the tropical *H. littoralis* was found to reach maximum pancratistatin content in October and a minimum in May. Generally, pancratistatin was accompanied by lesser yields of narciclasine, 7-deoxynarciclasine, and its *trans*-dihydro derivative.⁸³

4. Synthetic Modifications of Narciclasine and Its Congeners

4.1. Early Studies

The first investigation of narciclasine's chemistry was conducted by Piozzi et al.⁶³ and Mondon et al.¹⁰⁵ to confirm the structure of this important natural product and to generate a small library of analogues for biological structure–activity studies. Thus, narciclasine is selectively methylated at the phenolic hydroxyl with diazomethane in ethanol (**44** → **73**, Scheme 1). The resulting 7-methoxy derivative is easily oxidized with cold KMnO_4 to cotarnic acid (**74**). Acetylation of the three hydroxyl groups in **73** is achieved with acetic anhydride in pyridine, and triacetate **75** is converted back to **73** when reacted with $\text{Ba}(\text{OH})_2$ in methanol. Treatment of **44** with concentrated HCl leads to elimination of two molecules of water in ring C, providing aromatic analogue **48**, which is a natural product narciprimine, and it is found in narcissus bulbs in small quantities (section 2.3).

While these transformations were later used by other investigators to make narciclasine's derivatives, the yields for these reactions were not provided in the original report.⁶³ Furthermore, the positioning of the hydroxyl groups in narciclasine was erroneously established as in A, and thus, the structure of narciprimine was incorrectly deduced as B (Figure 15).

Mondon and Krohn¹⁰⁵ studied hydrogenation of the double bond in **44** and found that along with expected *trans* and *cis*

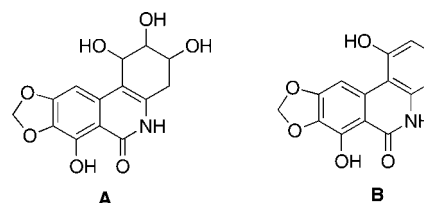
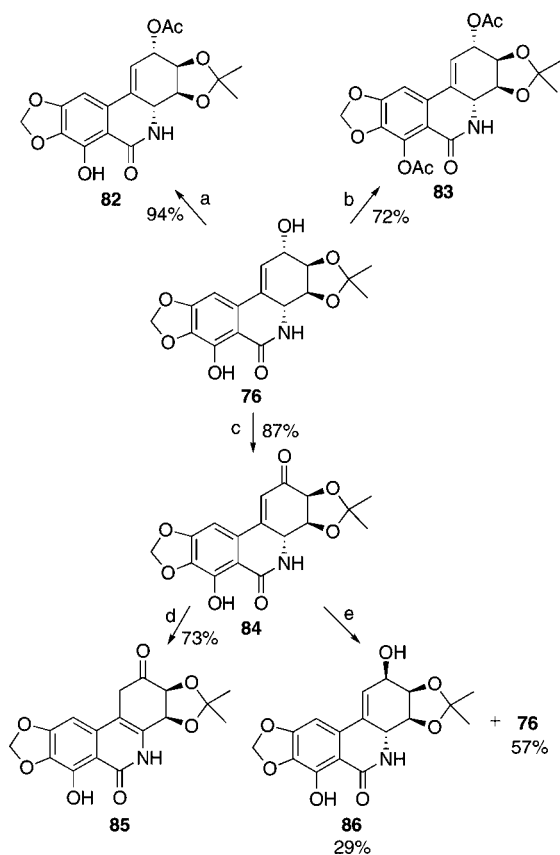


Figure 15. Incorrect original structure assignment of narciclasine (A) and narciprimine (B).

products **49** and **77**, the reaction results in the formation of isomeric compound **78**, named isonarciclasine. *trans*-Dihydronarciclasine **49** is a naturally occurring narciclasine analogue, and it was later isolated from *Z. candida* as was discussed in section 2.3. Selective functionalization of the hydroxyl groups in these products as well as narciclasine itself is achieved through the exclusive formation of C3,C4-acetonides **76** and **79–81** when the starting tetraols are treated with triethylorthoformate and *p*-TsOH in acetone. Fast acetylation of the C2-hydroxyl in acetonide **76** permits facile differentiation between the two remaining hydroxyl groups (Scheme 2). Although the reaction utilizes an excess of acetic anhydride in pyridine, it is stopped after 3 h of stirring at room temperature to give monoacetylated product **82** in excellent yield. When the components are allowed to react for 3 days, diacetyl acetonide **83** is formed. In addition, the C2-hydroxyl is smoothly oxidized to the corresponding ketone **84** with MnO_2 in THF. This compound is thermodynamically unstable and undergoes double bond isomerization to give **85**. Reduction of the keto function with sodium borohydride in ethanol proceeds nonselectively producing two isomeric secondary alcohols **86** and **76**. These can be easily separated; therefore, this transformation allows for the preparation of C2-*epi*-narciclasine.

Scheme 2^a

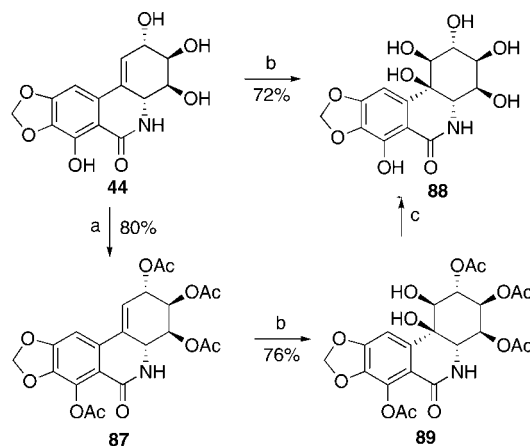
^a Reagents and conditions: (a) Ac₂O, py, 3 h. (b) Ac₂O, py, 3 days. (c) MnO₂, THF, 2.5 h. (d) Silica gel, THF. (e) NaBH₄, EtOH, 0.5 h, room temperature.

4.2. Studies by Pettit's Group and Semisynthesis of Pancratistatin from Narciclasine

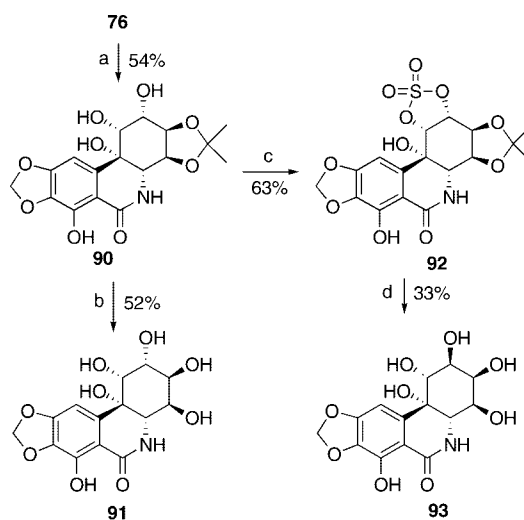
As part of an effort to develop a semisynthesis of pancratistatin from the more naturally abundant narciclasine, Pettit's group has extensively studied chemistry associated with narciclasine, generating a number of interesting analogues and eventually accomplishing such a relay synthesis of pancratistatin.

Thus, the double bond in narciclasine (**44**) as well as in its tetraacetate derivative (**87**) undergoes exclusive β -face dihydroxylation with catalytic OsO₄ in the presence of hydroquinone 4-chlorobenzoate (HQ) to give diols **88** and **89**, respectively (Scheme 3). Interestingly, the use of the pseudoenantiomeric catalyst hydroquinidine 4-chlorobenzoate (HQD) does not change this stereochemical outcome. Deacetylation of **89** with methanolic ammonia gives 10b-*R*-hydroxypancratistatin (**88**).^{106,107}

The investigators found that the facial preference of the dihydroxylation is affected by the protection pattern of narciclasine. Thus, narciclasine acetonide (**76**) undergoes attack by the osmium reagent from the α -face of the double bond preferentially, giving diol **90** and the corresponding β -diastereomer in a 2:1 ratio (Scheme 4). The acetonide group in **90** is smoothly removed with a solution of sulfuric acid in H₂O/THF to afford 10b-*S*-1-*epi*-pancratistatin (**91**). In an attempt to invert the configuration at C1 to that corresponding to natural pancratistatin, acetonide **90** was converted to cyclic sulfate **92** by treatment with thionyl chloride and subsequent oxidation of the intermediate sulfite

Scheme 3^a

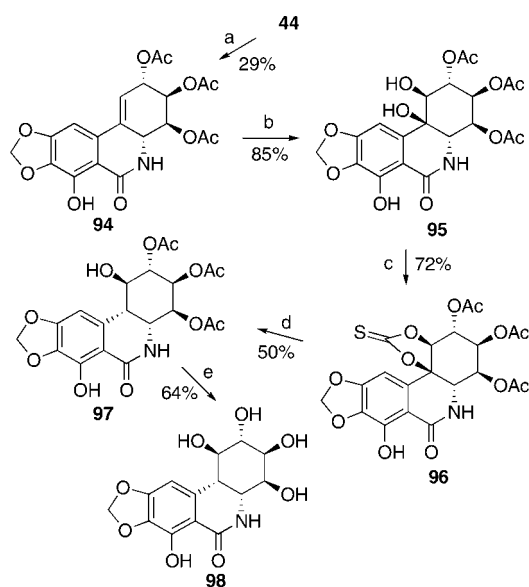
^a Reagents and conditions: (a) Ac₂O, py. (b) OsO₄ (cat.), NMO, HQ, DMF/H₂O. (c) NH₃/MeOH, CH₂Cl₂.

Scheme 4^a

^a Reagents and conditions: (a) OsO₄ (cat.), NMO, (DHQD)₂PHAL, DMF/H₂O. (b) H₂SO₄, THF/H₂O. (c) SOCl₂, Et₃N, THF; RuCl₃·3H₂O/NaIO₄, MeCN/CCl₄/H₂O. (d) PhCO₂H, Cs₂CO₃, DMF; H₂SO₄, THF/H₂O, 60 °C; K₂CO₃, MeOH.

with RuCl₃·3H₂O/NaIO₄. The ring opening with cesium benzoate, however, occurred instead at C2, affording after deprotection hexaol **93**.¹⁰⁷

Removal of the C10b tertiary benzylic alcohol by either hydrogenolysis or dehydration/hydrogenation in hexaol **88** was expected to furnish pancratistatin. All attempts, however, have not met with success. Instead, Pettit and co-workers investigated a radical deoxygenation approach utilizing a C10b-C1 cyclic thiocarbonate, with the expectation that the reaction would proceed with the correct regiochemistry due to stability of the tertiary benzylic radical at C10b.¹⁰⁸ Although acetylation of narciclasine with 3.25 equiv of Ac₂O results in a mixture of acetates, the desired triacetate **94** can be isolated by column chromatography in modest yield (Scheme 5). Highly selective β -dihydroxylation of the double bond in **94** provides triol **95**, which reacts with *N,N'*-thiocarbonyldiimidazole in 2-butanone to afford cyclic thiocarbonate **96** in good yield. Although successful, radical deoxygenation of **96** by heating with Bu₃SnH and AIBN in toluene gives the undesired *cis*-isomer **97**. Deacetylation of the latter compound results in 10b-*S*-pancratistatin (**98**), the *cis*-isomer of the natural isocarbostryl.

Scheme 5^a

^a Reagents and conditions: (a) Ac₂O (3.25 equiv), py. (b) OsO₄ (cat.), NMO, (DHQ)₂PHAL, DMF/H₂O. (c) *N,N'*-thiocarbonyldiimidazole, 2-butanone. (d) Bu₃SnH, AIBN, PhCH₃, 80 °C, 16 h. (e) K₂CO₃, H₂O/MeOH/CH₂Cl₂.

The successful strategy for the conversion of narciclasine to pancratistatin involves selective α -epoxidation of the double bond in diacetate **83**, followed by hydrogenolysis of C10b-C1 epoxide **99** (Scheme 6).¹⁰⁹ The reaction results in the formation of a mixture of four products, including the desired *trans* diol **101** in modest yield. The inversion of the C1 stereocenter is required, and this time it is achieved uneventfully by the formation of C1–C2 cyclic sulfate **104** and nucleophilic ring opening at C1 with cesium benzoate. Methanolysis of the benzoate ester in **105** gives pancratistatin (**51**) in good yield. Thus, pancratistatin is obtained from narciclasine in 10 steps and overall yield of 3.6%.

Another important investigation resulting in the synthesis of useful analogues of the natural isocarbostryril 7-deoxynarciclasine (**45**) and 7-deoxy-*trans*-dihydrnarciclasine (**50**, section 2.3) has also been reported by Pettit's group.⁸⁴ The study was prompted by a practical problem of separation of **45** and **50** after their isolation as a chromatographically inseparable mixture from *P. littorale* bulbs. It was hoped that various derivatization reactions might lead to easily separable compounds that could be converted back to the original isocarbostryrils or give rise to interesting analogues of these natural products. Thus, a fraction from *P. littorale*-containing triols **45** and **50** was treated with dimethoxypropane in DMF to give a mixture of corresponding acetonides **106** and **107** (Scheme 7). Protection of C2 hydroxyl in both compounds as TBS ether and reduction of the amide functionality with LiAlH₄ followed by removal of the silyl group gave easily separable by column chromatography acetonides **108** and **109** in respective yields of 53 and 18%. Removal of the acetonide protection led to the preparation of amine analogues **110** and **111** of the natural isocarbostryrils as their hydrochloride salts.

5. Biological Mechanism of Action

Preliminary biological evaluation of narciclasine, which was performed by Ceriotti immediately following its isolation from narcissus bulbs, already indicated a tremendous po-

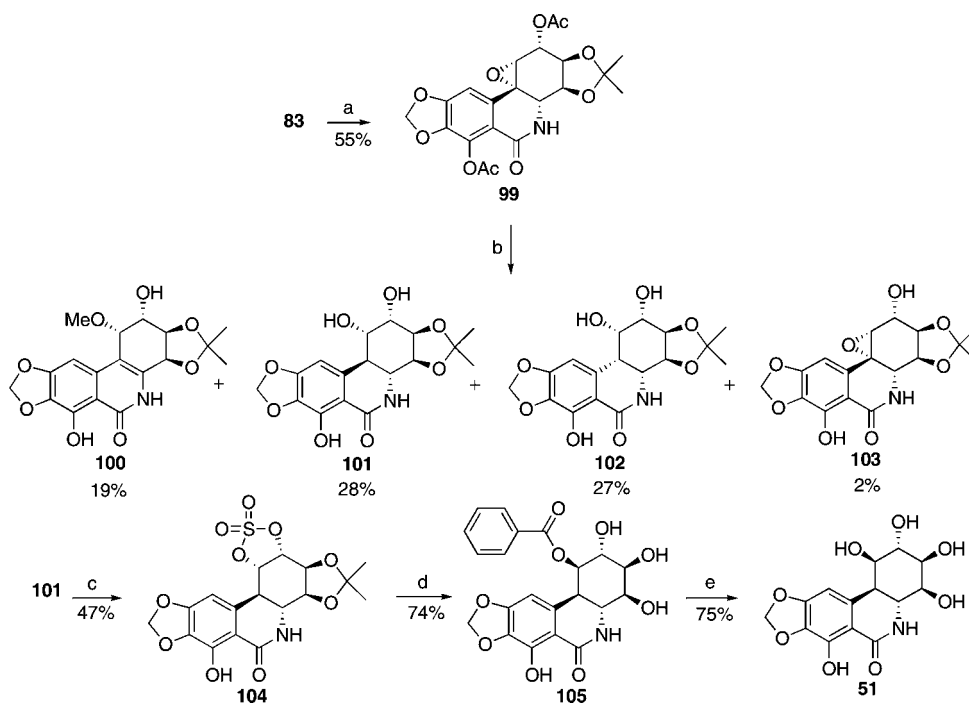
tential of this isocarbostryril to affect various biological systems.⁶⁴ Thus, Ceriotti reported that narciclasine strongly inhibited the growth of wheat grain radicles at very low concentrations and showed potent antimetabolic activity against the cells of sarcoma 180 in the ascites form when administered subcutaneously in low dosages. Studies by a number of laboratories, most prominently by Spanish investigators at the Instituto de Biología in Madrid, established that these as well as many other biological activities of narciclasine can be attributed to its potent inhibition of eukaryotic ribosomal protein biosynthesis. Carrasco et al. first reported that narciclasine inhibited poly U-directed [¹⁴C]phenylalanine incorporation in yeast polysomes and rabbit reticulocyte (immature red blood cells) ribosomes.¹¹⁰ The authors further went on to show that the isocarbostryril inhibited the association of both [³H]anisomycin and [¹⁴C]trichodermin with both yeast and rabbit reticulocyte ribosomes. Anisomycin (Figure 16), an antibiotic produced by *Streptomyces griseolus*, and trichodermin, an antifungal metabolite, are both known to inhibit peptide bond formation by eukaryotic ribosomes by association with the peptidyl transferase catalytic center integrated with the 60S ribosomal subunit.

Furthermore, Jimenez et al. showed that the mutant *Saccharomyces cerevisiae* TR₁ strain, which possesses a ribosomal alteration in the peptidyl transferase center of the 60S subunit and is resistant to both anisomycin and trichodermin, shows cross-resistance to narciclasine. While the isocarbostryril inhibits the uptake of [³H]leucine into the proteins of the wild-type *S. cerevisiae* strain Y166, similar concentrations of narciclasine fail to produce the same effect with trichodermin and anisomycin-resistant cells.^{111,112} These and additional experiments prompted the investigators to conclude that narciclasine interacts with the 60S eukaryotic ribosome subunit and inhibits peptide bond formation step in eukaryotic protein biosynthesis.

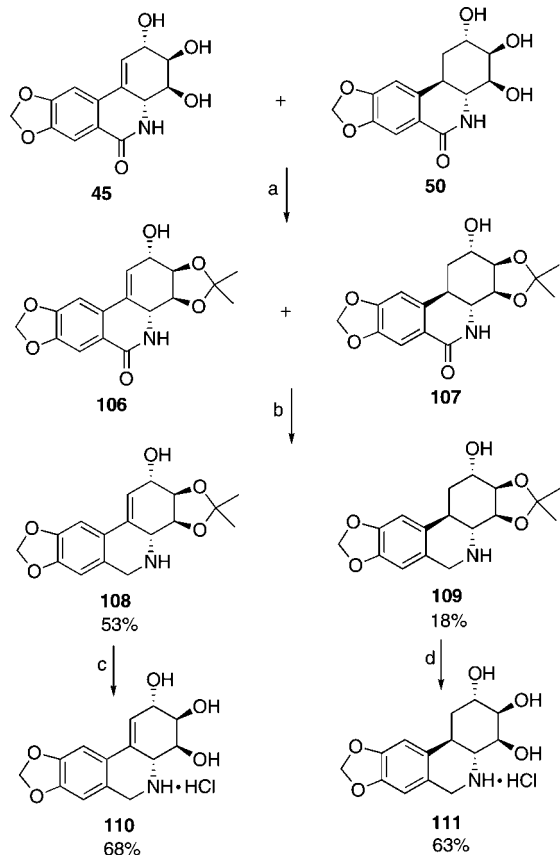
The same investigators further proceeded to evaluate comparatively the effects of narciclasine and 17 Amaryllidaceae alkaloids on (i) HeLa cell growth and (ii) DNA, RNA, and protein syntheses in intact Krebs ascites cells.¹¹³ DNA, RNA, and protein syntheses were studied by incorporation of [¹⁴C]thymidine, [¹⁴C]uridine, and [¹⁴C]proline, respectively. Narciclasine (**44**) and five of the alkaloids, namely, lycorine (**3**), haemanthamine (**5**), dihydrolycorine (**39**), pseudolycorine (**38**), and pretazettine (**7**), exhibit potent growth-inhibitory activity against HeLa cells (Table 3).

The same six compounds inhibit *in vivo* protein synthesis and to a much lesser extent DNA synthesis, while RNA synthesis is unaffected. The concentration ranges required for the inhibition of protein synthesis are in good agreement with the MIC values defined as concentrations required for complete inhibition of HeLa cell growth. The authors proposed that the weak inhibition of the DNA synthesis is a secondary effect of the potent inhibition of protein synthesis, since translation is required for DNA synthesis. These results further corroborate the idea that the inhibition of protein biosynthesis is the primary mode of action of narciclasine as well as a number of other Amaryllidaceae plant constituents in eukaryotic cells.

To obtain direct evidence that narciclasine indeed interacts with eukaryotic ribosomes, Baez et al. prepared a tritium-labeled narciclasine derivative (Scheme 8).¹¹⁴ Although the site of tritium incorporation in narciclasine was not reported by the investigators, it is reasonable that under the described

Scheme 6^a

^a Reagents and conditions: (a) MCPBA, phosphate buffer. (b) H₂, 10% Pd/C; K₂CO₃, MeOH/H₂O. (c) SOCl₂, Et₃N, THF; RuCl₃·3H₂O/NaIO₄, MeCN/CCl₄/H₂O. (d) PhCO₂H, Cs₂CO₃, DMF; H₂SO₄, THF/H₂O, 70 °C. (e) K₂CO₃, MeOH.

Scheme 7^a

^a Reagents and conditions: (a) Me₂CH(OMe)₂, *p*-TsOH, DMF. (b) TBSCl, imidazole, DMF; LiAlH₄, ether; TBAF, THF. (c) Aqueous H₂SO₄, THF/CH₂Cl₂; NaHCO₃; HCl, MeOH. (d) HCl, MeOH.

reaction conditions the electrophilic aromatic substitution takes place to give C10-labeled compound.

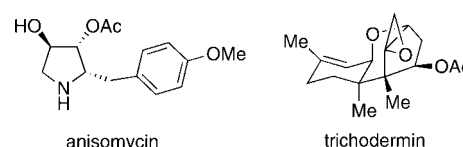


Figure 16. Structures of anisomycin and trichodermin.

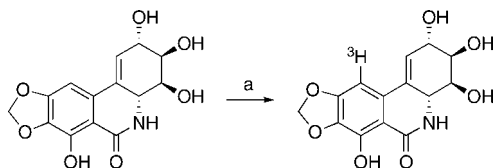
Using [³H]narciclasine, the investigators confirmed that the isocarbostyryl specifically interacts with 60S ribosome subunits of both *S. cerevisiae* and rabbit reticulocytes, but not with 40S subunits. Furthermore, narciclasine failed to bind to the ribosomes from *E. coli*, explaining an earlier observation of narciclasine's protein synthesis inhibition specifically in eukaryotic cells and not in those of *E. coli*.¹¹⁰

[³H]Narciclasine was used by Baez and Vazquez to perform a structure–activity relationship study of narciclasine derivatives.¹¹⁵ More specifically, various synthetic narciclasine derivatives were assayed for their propensity to inhibit the poly U-directed poly[¹⁴C]phenylalanine synthesis by yeast ribosomes and the activity patterns were correlated with the ability of these compounds to inhibit the binding of [³H]narciclasine to yeast ribosomes (Table 4).

Thus, *trans*-dihydronarciclasine (**49**), *trans*-dihydronarciclasine acetonide (**79**), and isonarciclasine (**78**) inhibit both polyphenylalanine synthesis and [³H]narciclasine binding, suggesting that these compounds interact with the same ribosomal site as narciclasine. It is also possible that less potent *cis*-dihydronarciclasine (**77**) and *O*-methylnarciclasine (**73**) have the same binding sites as narciclasine, but their affinities for the ribosome are much lower since they have little inhibitory effect on [³H]narciclasine binding. Of interest is the significant difference in activities of *trans*- and *cis*-dihydronarciclasines as well as the fact that methylation of the 7-OH group in narciclasine diminishes the activity but does not completely abolish it. Not shown in Table 4 due to complete lack of activity in both assays are narciclasine

Table 3. Comparative Biological Evaluation of Selected Amaryllidaceae Constituents Used in the Study by Jimenez et al.

compound	μM			
	MIC	IC ₅₀		
	HeLa cell growth inhibition	protein synthesis inhibition	DNA synthesis inhibition	RNA synthesis inhibition
44	0.1	0.07	~1	> 1
39	100	300	~1000	> 1000
3	6	4	20	> 20
7	1	3	> 100	> 100
5	4	1	~10	> 10
38	25	8	30	> 100

Scheme 8^a

^a Reagents and conditions: (a) $^3\text{H}_2\text{O}$, Pd/C, 60 °C, 23 h, sealed ampoule; coevaporation with ethanol (five times).

acetone (76), *trans*-dihydronarciclasine tetracetate, *cis*-dihydronarciclasine tetracetate, *O,N*-dimethylnarciclasine, 7-deoxynarciclasine triacetate, and *O*-methyl-*cis*-dihydronarciclasine.

6. Effects of Narciclasine and Its Congeners on Plant Physiology

6.1. The “Vase Effect”

Ornamental flower growers know that placing cut daffodils in a vase with other cut flowers has a negative effect on the flower quality and significantly shortens their vase life. Barendse provided more specific descriptions of such visual observations.¹¹⁶ For example, when 10 cut roses (var. Baccara) were placed in a vase together with 10 daffodils (var. Carlton) for 4 h at 2 °C, the rose flowers desiccated, petals changed from red to bluish red, and the leaves abscised. Daffodils inhibited flower opening in Iris (var. Ideal), freesia (var. Golden Melody), and anemones just after a short exposure in the vase. Tulips (var. Parade) underwent stem bending, precocious tepal bluing, and leaf shriveling. Furthermore, a common horticultural practice for the cultivation of narcissus flowers involves the removal of the old scales and roots of the bulbs followed by introduction of cuts on the bulbs before immersing them into water. The mucilage that leaches out from the cuts is constantly removed by frequent changing of water. This procedure leads to sprouting and raises a speculation that an inhibitor is present in the mucilage of the narcissus bulbs. van Doorn studied the daffodil “vase effect” on roses and tulips in some detail (Table 5) and concluded that the physiological reasons for shortening of the vase lives of these two flowers were different.¹¹⁷ Daffodils or their stem mucilage negatively affect water uptake in roses and result in water stress symptoms (wilting, lack of flower opening) in these flowers. In contrast, the water uptake by tulips was only little affected, and instead of water stress symptoms, precocious leaf yellowing was observed. The latter effect was ascribed by the investigator to the toxic properties of the alkaloids in daffodil mucilage.

In a recent study, van Doorn et al.¹¹⁸ obtained evidence pointing to narciclasine, a constituent of daffodil mucilage, as a primary component responsible for the daffodil “vase

effect”. The investigators studied the effect of delayed senescence manifested by Iris (var. Hollandica) flowers when the latter are placed in a vase containing a Carlton daffodil stem. Senescence in Iris flowers is normally characterized by inward rolling of the flag tepals and color change from purple to yellowish white, followed by desiccation. The exposure of the Iris flowers to a daffodil stem results in remarkable delay of tepal senescence; the flowers eventually wilt while retaining the color. The same effect was observed when either daffodil mucilage (0.3 mL/L) or purified narciclasine (0.6 μM) was added to the water. The investigators hypothesized that narciclasine may inhibit the synthesis of enzymes required for the senescence-associated degradation processes. Indeed, narciclasine significantly attenuates the increase in protease activity and inhibits lipid breakdown in tepal edges of cut Iris flowers.¹¹⁸

6.2. Plant Growth-Inhibitory Properties

A number of reports describe plant growth-inhibitory properties of narciclasine. It inhibits the growth of coleoptiles of wheat embryos and significantly decreases the number of mature spikelets of *Lolium temulentum*.¹¹⁹ The isocarbostryl interferes with the elongation growth of *Avena* coleoptiles rice seedlings,⁶⁷ etiolated pea epicotyls,¹²⁰ and cress roots¹²¹ and inhibits the callus growth of cultured tobacco piths⁶⁷ as well as radicle growth of wheat.⁶⁴

Inhibition of protein biosynthesis by narciclasine could account for most, if not all, of these and related observations. The studies of antagonistic effects of narciclasine on auxin action in plants are particularly revealing in this respect. Various auxin bioassays in *L. radiata*¹²² and *N. pseudonarcissus*¹²³ failed to produce growth-promoting effect, and this may be attributed to the presence of a protein inhibitor, such as narciclasine, in the tissue extracts. Indeed, Kang et al. reported that narciclasine strongly suppresses auxin-induced ethylene production in mung bean (*Vigna radiata* Wilczek) seedlings, while potently inhibiting [¹⁴C]leucine incorporation into protein in the tissue.¹²⁴ The investigators further showed that narciclasine does not inhibit ethylene synthesis from its immediate precursor, 1-aminocyclopropane-1-carboxylic acid (ACC), during short-term incubation, but the activity of ACC synthase and, therefore, the amount of ACC in the tissue treated with indole-3-acetic acid (IAA) are significantly diminished by narciclasine. Taken together, these results suggest that the auxin-induced ethylene formation is not due to a specific interaction of narciclasine with auxin but rather results from the general inhibition of protein synthesis by narciclasine. A similar conclusion was made by Sasse et al., who investigated the inhibitory effect of narciclasine on fusicoccin and IAA-induced growth of dwarf pea (*Pisum sativum* L.) hooks and tips.¹²⁵ Narciclasine completely suppresses IAA-induced growth, whereas fusi-

Table 4. SAR Study of Narciclasine Derivatives

compound	concentration	[¹⁴ C]Phe incorporation	concentration	[³ H]narciclasine binding
	mM	% control	mM	% control
44	0.1	6	N/A	N/A
	1	0		
49	0.1	8	0.01	25
	1	5	1	4
79	0.1	8	0.01	44
	1	6	1	5
77	0.1	67	0.1	96
	1	52	1	90
73	0.1	49	0.1	95
	1	43	1	70
78	0.1	21	0.1	68
	1	12	1	16

Table 5. Effect of Daffodils (var. Carlton) and Their Stem Mucilage (1% v/v) on the Vase Life and the Rate of Water Uptake of Roses (*Rosa hybrida* Sonia) and Tulips (*Tulipa gesneriana* Apeldoorn)

treatment	vase life (days)	water uptake (mL/stem/h)
one rose per vase	~11	~9
two roses per vase	~10	~9
one rose, one daffodil	~3	~4
one rose, daffodil mucilage	~3	~3
one tulip per vase	~7	~5
two tulips per vase	~7	~5
one tulip, one daffodil	~4	~4
one tulip, daffodil mucilage	~4	~4

coccin-induced growth is only partially inhibited. Because it is known that the IAA effect on plant growth fully depends on protein synthesis and fusicoccin action is only partially dependent on it, general inhibition of protein synthesis by narciclasine in this plant tissue provides a satisfactory explanation for these observations.

6.3. Other Effects

Other noteworthy effects of narciclasine on plant physiology include inhibition of auxin transport in *Citrullus edulis* seedlings,¹²⁶ blocking of chlorophyll biosynthesis and chloroplast formation in wheat (*Triticum aestivum*) seedlings,¹²⁷ and possible phytoalexin action.⁸¹ Both narciclasine and 7-deoxynarciclasine were found to be main antifeedants in the bulbs of *L. radiata*.¹²⁸ Narciclasine appeared to be the most potent inhibitor of ascorbic acid biosynthesis in vivo among 40 Amaryllidaceae alkaloids and their synthetic derivatives tested in potato tubers, as was reported by Evidente et al.¹²⁹

Lastly, most of narciclasine congeners, particularly 7-deoxynarciclasine, pancratistatin, and 7-deoxypancratistatin, have been shown to have similar plant growth-inhibitory properties to those of narciclasine. In this connection, growth-stimulatory effects of 2-*O*- β -glucosides of narciclasine (kalbreclatine, **46**) and pancratistatin (pancratiside, **53**) are intriguing.⁷¹ Thus, Ghosal et al. detailed such contrasting behavior for the pancratistatin/pancratiside pair with respect to the germination of seeds and the development of roots of *Zephyranthes flava*. While under normal incubation conditions 40–50% of the control seeds were germinated after 36 h, 100% of the pancratiside-treated (0.1 mM) and none of the pancratistatin-treated seeds were germinated. Similarly, growth and elongation of roots of *Z. flava* were enhanced by pancratiside (235 and 177% over control), while pancratistatin inhibited these processes (70 and 95% over

control).⁷¹ Apparently, such a phenomenon is general for these classes of Amaryllidaceae constituents, since similar observations were reported for lycorine/lycorine-1-*O*- β -glucoside pairs.^{130,131} Further investigation of these effects will undoubtedly shed more light on plant growth regulatory mechanisms.

7. Effects of Narciclasine and Its Congeners Potentially Beneficial to Human Health

7.1. Anticancer Activities

Early studies by Ceriotti on biological activities of narciclasine revealed that this constituent of the daffodil bulbs possesses potent antimetabolic properties against the highly malignant mouse sarcoma cells (Sarcoma 180) passaged in the ascites form.⁶⁴ Narciclasine was administered by intraperitoneal or subcutaneous injections or via oral route. Subcutaneous injection was most effective and resulted in complete disappearance of mitoses after 4 h of administration at the doses of 0.9 mg/kg. In addition, an LD₅₀ value of 5 mg/kg in mice was established for the subcutaneous injection administration route. Working independently at about the same time, Okamoto et al. reported toxic effects of narciclasine and 7-deoxynarciclasine toward the cells of Ehrlich Carcinoma, with the former isocarbostryl being more potent.⁶⁷ Mondon and Krohn reported the first SAR investigation of narciclasine and its derivatives by assessing their toxicities to HeLa and HEP₂ cell lines as models of human cervical and laryngeal carcinomas, respectively.¹⁰⁵ Narciclasine, *O*-methylnarciclasine (**73**), and *trans*-dihydronarciclasine (**49**) were most potent exhibiting toxicities at concentrations of 0.1 μ g/mL. Significantly less potent were *cis*-dihydronarciclasine (**77**) and *O,N*-dimethylnarciclasine, while narciclasine acetone (**76**) and isonarciclasine (**78**) were found to be inactive. These SAR data are in good agreement with the results of Baez and Vazquez (Table 4, vide supra)¹¹⁵ of the suppression of poly U-directed poly[¹⁴C]phenylalanine synthesis as well as inhibition of binding of [³H]narciclasine to yeast ribosomes. The only exception is the total lack of toxicity of isonarciclasine (**78**) to the intact cells, although it is quite potent in the cell free ribosomal assays. Membrane permeability appears to be an important consideration for comparing the outcomes of these two assays.

Many bioactivity-guided isolations of narciclasine congeners from their respective plant sources utilized murine P-388 lymphocytic leukemia in vitro and in vivo (PS system) bioassays.^{132,133} The efficacies of the purified natural products were also initially assessed using this cell line. Although

Table 6. Results of Comparative Anticancer Evaluation of Narciclasine and Related Isocarbostryrils in the NCI 60 Cell Line Screen

compound	mean GI ₅₀ (μ M)	compare correlation coefficient
narciclasine (44)	0.016	0.90
7-deoxynarciclasine (45)	0.15	0.90
<i>trans</i> -dihydronarciclasine (49)	0.013	0.92
7-deoxy- <i>trans</i> -dihydro- narciclasine (50)	0.068	0.89
pancratistatin (51)	0.091	1
<i>cis</i> -dihydronarciclasine (77)	3.8	<0.6
7-deoxy- <i>cis</i> -dihydro- narciclasine (112)	95.5	<0.6
isonarciclasine (78)	11.8	<0.6

the in vitro potencies reported in the literature vary, narciclasine (**44**), 7-deoxynarciclasine (**45**), *trans*-dihydronarciclasine (**49**), 7-deoxy-*trans*-dihydronarciclasine (**50**), and pancratistatin (**51**) have all registered GI₅₀ values as low as 0.01 μ g/mL.^{70,74,75,84} In addition, pancratistatin (**51**) was effective in vivo against this cancer (38–106% life extension at 0.75–12.5 mg/kg) as well as murine M-5076 ovary sarcoma (53–84% life extension at 0.38–3.0 mg/kg).^{70,73} These initial promising data warranted the evaluation of the above five narciclasine congeners as well as synthetic *cis*-dihydronarciclasine (**77**), 7-deoxy-*cis*-dihydronarciclasine (**112**, obtained by hydrogenation of **45**),¹³⁴ and isonarciclasine (**78**) in the NCI 60 human cancer cell line in vitro screen.¹³⁵ The GI₅₀ values, as reported Pettit et al.,⁷² are averaged across the entire 60 cell line panel and are given in Table 6. Table 6 also lists the “Compare Correlation Coefficients”, which are generated by a computerized pattern recognition algorithm and serve as an indication of similarities in differential cellular sensitivities or characteristic fingerprints for each compound.¹³⁵ The synthetic analogues **77**, **78**, and **112** are only weakly active or inactive in this in vitro screen, while the natural isocarbostryrils (**44**, **45**, **49**–**51**) exhibit nanomolar growth-inhibitory potencies. These results are consistent with the data from the SAR studies of the inhibition of eukaryotic protein biosynthesis (Table 4) and provide further support for this mechanism of action for narciclasine and its congeners in eukaryotic cells. Furthermore, the “Compare Correlation Coefficients” show that the differential cellular response profiles are very similar for these five compounds, further corroborating a uniform mode of action. Importantly, the differential cellular sensitivity profile associated with these isocarbostryrils does not show good correlation with any of the known anticancer agents. The melanoma subgroup of cancer cell lines is the most responsive, while selected cell lines from within such subpanels as NCS lung, colon, brain, and renal are more than 1000 times more responsive than the least sensitive cell lines within these subpanels. Pettit and co-workers concluded their analysis of NCI screening data with the following statement: “Indeed, it seems likely that pancratistatin and related *trans*-dihydrocarbostryrils, as well as narciclasine, constitute the most important antineoplastic components of Amaryllidaceae species used in the folk medical treatment of cancer.”⁷²

Additionally, potent cytotoxic/antitumor activities were reported for narciclasine-4-*O*- β -D-glucopyranoside (**47**).⁷⁷ Using the brine shrimp (*Artemia salina*) assay, Abou-Donia et al. obtained the LD₅₀ value of 0.88 μ g/mL for **47** (LD₅₀ = 0.29 μ g/mL was obtained for **44** in this assay). Both **44** and **47** exhibited antitumor activity in the potato disk antitumor assay by inhibiting crown gall tumor initiation (62% inhibition for **44**, 53% inhibition for **47**). Also, C1-

esterified naturally occurring pancratistatin derivatives **55** and **56** showed cytotoxicities comparable to pancratistatin itself when tested against HeLa, KB, and P-388-D1 (models for human cervical and epidermoid carcinomas as well as murine lymphocytic leukemia, respectively) cell lines.⁷⁹ In addition, both nonglycosylated (**55**) and glycosylated (**56**) pancratistatin C-1 esters, as well as pancratistatin itself, were later shown to cause cell cycle arrest and apoptosis in promyelocytic leukemia HL-60RG cells.¹³⁶ 7-Deoxypancratistatin (**52**), originally reported to have plant growth-inhibitory properties,⁷¹ was later evaluated against human cancer cell lines and found to be ca. 10 times less potent than its 7-hydroxy congener.¹³⁷

Recently, Pandey and co-workers reported the results of investigations of the effects of pancratistatin on normal and cancer cells, providing evidence supporting selective toxicity of this isocarbostryril to cancer cells. Thus, pancratistatin was found to selectively induce apoptosis using nongenomic targets in the Jurkat (model for human T-cell leukemia) cell line as opposed to normal nucleated blood cells.¹³⁸ While pancratistatin is cytotoxic at submicromolar concentrations to a number of human cancer cell lines, normal human fibroblasts (NHF) and human umbilical vein endothelial cells (HUVEC) are unaffected by the same treatments. In contrast, such chemotherapeutic drugs as paclitaxel and etoposide show toxicity to both of these noncancerous cell lines.¹³⁹ Finally, the authors speculated that pancratistatin may take advantage of the differences between mitochondria in cancerous and noncancerous cells to selectively induce apoptosis in the former cell types.¹⁴⁰ Further studies will be necessary to understand the nature of this effect in detail and reconcile these findings with the inhibition of protein biosynthesis as the established mode of action for narciclasine and its congeners. These experiments, however, bode well for the clinical development of pancratistatin, narciclasine, and their analogues.

Pettit and co-workers have tested all of the synthetic intermediates shown in Schemes 3–7, which represent a large selection of analogues accessible from the natural isocarbostryrils against murine P-388 lymphocytic leukemia cells as well as a minipanel of human cancer cell lines including pancreas (BXPC-3), breast (MCF-7), CNS (SF268), NCS lung (NCI-H460), colon (KM20L2), and prostate (DU-145) in vitro cancer models. All C10b-hydroxyanalogues of pancratistatin, including those with *cis* (**88**) and *trans* (**91**, **93**) B/C ring junctions, are significantly less potent than the natural compound or are inactive.¹⁰⁷ The C10b-epimer of pancratistatin (**98**), possessing the *cis* B/C ring fusion, is less active than the natural isocarbostryril by a factor of 10.¹⁰⁸ An intriguing discovery is the high potency of C1-benzoate ester pancratistatin derivative (**105**), which exceeds that of pancratistatin by 10–100 times depending on the cell type.¹⁰⁹ Whether the benzoate moiety enhances the activity by facilitating the intracellular delivery and is subsequently hydrolyzed within cells or by serving as an important part of the cytotoxic pharmacophore is not clear. Further studies are anticipated. Finally, replacing the amide functionality by an amino group in **110** and **111** reduces the toxicities 10–100 times relative to the natural isocarbostryrils.⁸⁴

Synthetic efforts by many research groups, aimed at preparation of analogues inaccessible from the natural isocarbostryrils, have been invaluable in defining the cytotoxic pharmacophore(s) associated with these compounds to a much greater extent. The analogues, prepared from com-

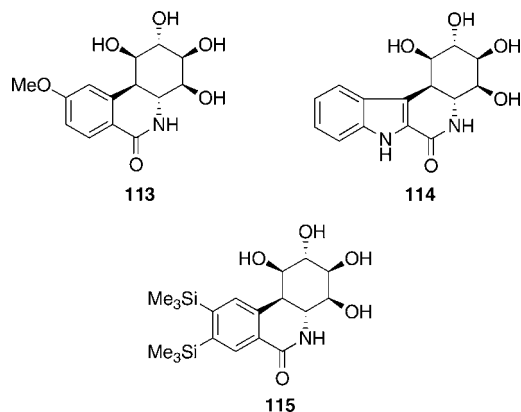


Figure 17. Ring A analogues.

mercially available starting materials, can be divided into three categories based on whether the major structural differences with the natural compounds are located in rings A, B, or C.

As has been already discussed, removal of the 7-hydroxyl group in ring A as in 7-deoxypancratistatin reduces the potency of pancratistatin 10-fold.¹³⁷ The inspection of the NCI 60 cell line primary screening data in Table 6, as well as the results from independent testing by Pettit's group,⁸⁴ indicates that the similar effect is present in narciclasine/7-deoxynarciclasine and *trans*-dihydronarciclasine/7-deoxy-*trans*-dihydronarciclasine pairs. Furthermore, Hudlicky et al. have synthesized a singly oxygenated ring A analogue of pancratistatin (**113**, Figure 17)¹⁴¹ and showed that removal of another oxygen in ring A of pancratistatin further lowers the potency 10-fold relative to 7-deoxypancratistatin (100-fold relative to pancratistatin).

Bioisosteric replacement of methylenedioxybenzene moiety with the indole ring is a common practice in drug design;¹⁴² therefore, Hudlicky's group performed an ab initio computational comparative analysis of such β -carboline-1-one mimic of pancratistatin (**114**).¹⁴³ Indeed, the results of the computational study were encouraging showing a significant amount of overlapping parameters, including favorable geometries and electrostatic potential distribution, between **114** and pancratistatin containing methylenedioxybenzene moiety. The investigators developed an efficient nine-step synthesis of **114**.^{143,144} Disappointingly, the indole-containing compound showed only weak activity against the murine P388 cells and was completely inactive against a panel of human cancer cell lines. Finally, bis-silylated ring A analogue **115**, an intermediate resulting from the synthetic efforts by Hudlicky's group,^{137,145} was found to be inactive as well.

The stereochemistry of the B/C ring junction appears to be an important element affecting the analogues' potency. *Trans* ring junction as in natural carbostyrils **49–52** or the sp^2 character of the C10b position as in **44** or **45** seem to be critical for cytotoxicity. The inversion of the C10b stereocenter resulting in the *cis* B/C fusion stereochemistry leads to reduction or complete loss of potency. This has been demonstrated with the synthetic analogues *cis*-dihydronarciclasine (**77**) and 7-deoxy-*cis*-dihydronarciclasine (**112**) in the 60 cancer cell line screen (Table 4) as well as the C10b-epimer of pancratistatin (**98**) as has been discussed above. Furthermore, Hudlicky et al. synthesized the C10b-epimer of 7-deoxypancratistatin (**116**, Figure 18) and showed that it is inactive as well.¹⁴⁶ It is of interest that none of these

cis-analogues has been isolated from the natural sources, although many of the isolations are bioactivity-guided and these compounds could have been easily missed by natural product chemists. To date, no variation in ring B has resulted in improved activity. Lactone analogues of 7-*O*-methylnarciclasine (**117**) and 7-deoxynarciclasine (**118**), synthesized by Chapleur and co-workers, were found to be inactive.¹⁴⁷ As was mentioned above, analogues **110** and **111**, lacking the amide functionality in ring B, are significantly less potent than the parent isocarbostyrils. In this connection, the *N*-tosyl analogue (**119**), containing significant structural variations in both rings A and B, was found to have low micromolar growth-inhibitory potencies against a panel of human cancer cell lines, as was reported by Hudlicky and co-workers.¹³⁷ Because of such profound differences in structure, it is likely that this compound works through a different mechanism than the parent isocarbostyril. Furthermore, Chapleur and co-workers reported a synthesis of seco-analogues **120** and **121**, lacking the C10a-C10b bond.¹⁴⁸ The compounds were shown to be inactive, demonstrating the importance of the rigid geometry with the intact ring B. Fessner and co-workers described an interesting biocatalytic synthesis of a carbohydrate type analogue **122**, containing variations in both rings B and C.¹⁴⁹ The results of biological testing of this compound have not been reported, but because the lactone ring B analogues are inactive, it will be hard to assess the effect of the endocyclic oxygen in ring C on cytotoxic activity. Lastly, Kornienko and co-workers reported the preparation of arylcyclitol libraries (**123–128**), which lack the lactam moiety but incorporate the C10a-C10b bond with the correct configuration.^{150,151} The compounds were found to be inactive, results that complement the findings of Chapleur on the importance of the intact ring B.

7-Deoxy-*trans*-dihydronarciclasine (**50**) is a nanomolar cancer cell growth-inhibitory compound, and it represents the natural isocarbostyril with the fewest number of the hydroxyl groups. Several research groups have invested much effort to elucidate the minimum requirement for cytotoxicity in terms of the oxygen functionality in the molecule. Compounds **129** and **130** (Figure 19), completely lacking any hydroxyl groups in ring C, were prepared but unfortunately not evaluated for biological activity.^{152–154} Similarly, Banwell and co-workers prepared 2-deoxy analogue of 7-deoxynarciclasine (**131**) but did not report any biological data for this interesting test compound.¹⁵⁵

The synthesis and biological evaluation of compounds **132–134** by McNulty and co-workers^{156,157} as well as **135** by Hudlicky and co-workers¹⁴¹ have clearly indicated the importance of having at least three hydroxyl groups in ring C for potent cytotoxic activity. Compounds **132** and **133** were found inactive, **134** is on average 2–3 orders of magnitude less potent than pancratistatin in the NCI 60 cell line screen, and **135** only showed low micromolar growth-inhibitory potency against the murine P388 cell line but was completely inactive against a panel of human cancer cell lines. Unfortunately, no biological testing data were provided for compound **136**, synthesized by Pandey et al.¹⁵⁸ It would be instructive to learn how the transposition of the hydroxyl group from C2 (as in **50**) to C1 (as in **136**) affects the cytotoxicity. Lastly, diol **137** and tetraacetate **138**, the latter being C2-epimeric to 7-deoxypancratistatin, have been synthesized both in racemic form by DeShong's¹⁵⁹ and Alonso's¹⁶⁰ groups, respectively. However, no biological activity data were provided. Finally, Keck's and Hudlicky's

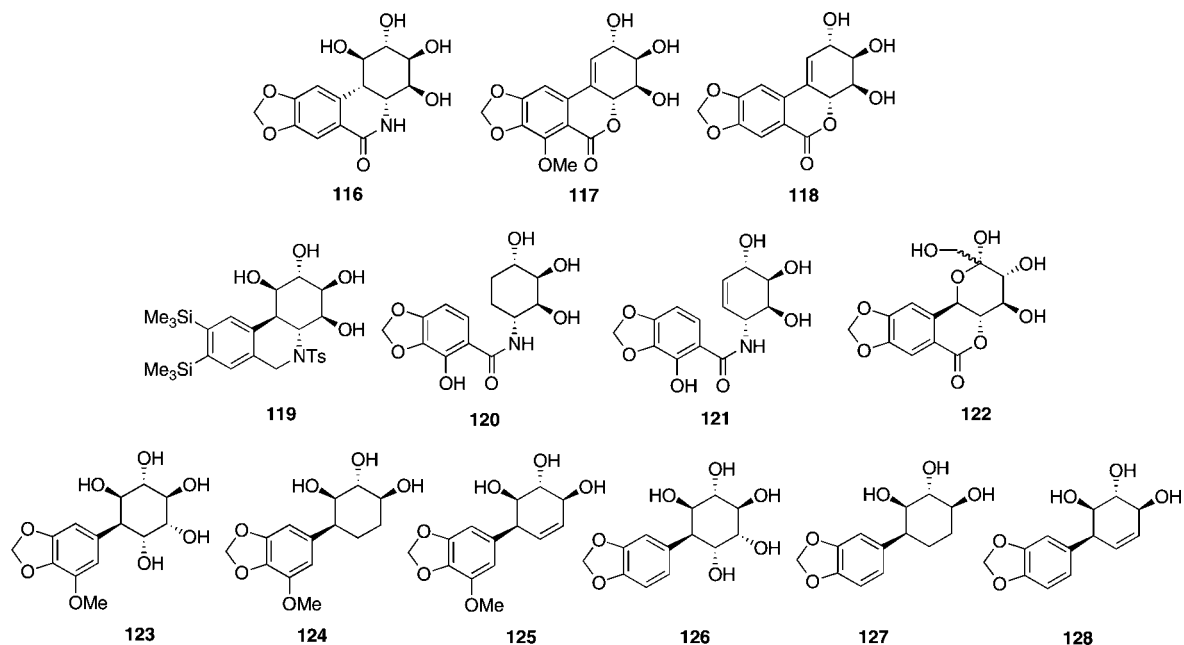


Figure 18. Ring B analogues.

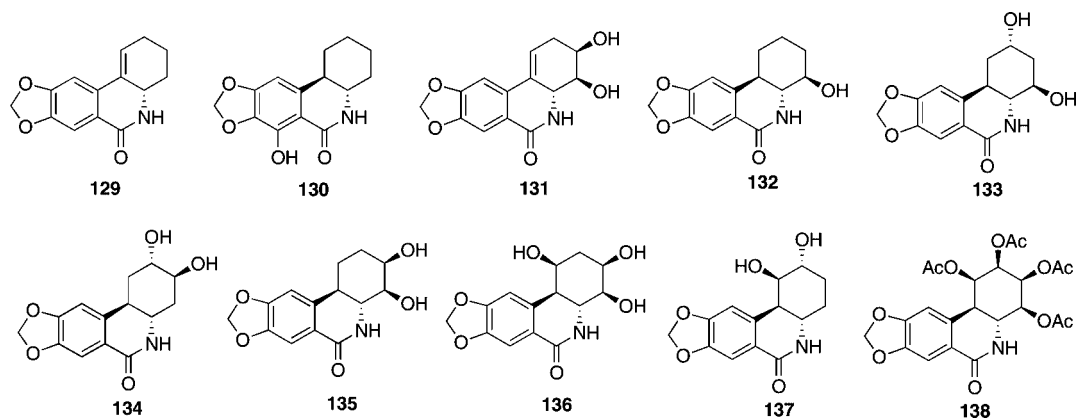
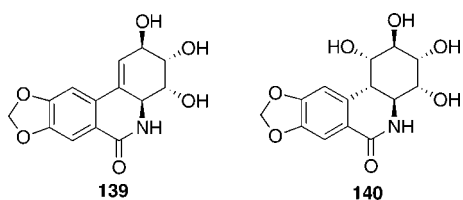


Figure 19. Ring C analogues.

Figure 20. Structures of *ent*-7-deoxynarciclasine (**139**) and *ent*-7-deoxypancratistatin (**140**).

groups prepared unnatural optical antipodes of 7-deoxynarciclasine (**139**) and 7-deoxypancratistatin (**140**), respectively (Figure 20).^{161–163}

While the results of biological testing of *ent*-7-deoxynarciclasine have not yet been disclosed, rather surprisingly, *ent*-7-deoxypancratistatin exhibits cytotoxic potency, which is only 10-fold lower than the corresponding natural enantiomer, when the latter compound was evaluated in a minipanel of human cancer cell lines.¹⁴⁶ Interestingly, the inspection of the stereochemical relationships between different Amaryllidaceae anticancer constituents reveals the pseudoenantiomeric nature of some of them. Thus, relative configurations of the four stereocenters in the structure of lycorine (**3**, Figure 1) are similar to those of pancratistatin (positions C2, C1, C10b, and C4a), while being opposite in

the absolute sense. Given the fact that the lycorine and narciclasine as well as their congeners are believed to exert their growth-inhibitory properties by the inhibition of protein biosynthesis in eukaryotes, preparation and biological testing of the optical antipodes of narciclasine, pancratistatin, and *trans*-dihydrnarciclasine are intriguing with respect to further elucidation of the origin of the activity associated with these molecules as well as development of their analogues that could be less toxic to normal cells.

7.2. Antiviral Activities

Narciclasine and its natural and synthetic analogues were evaluated for antiviral activity in the drug discovery program of the U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID).^{164,165} The analogues were tested against tropical RNA viruses, which cause hemorrhagic, encephalitic, or febrile disease in large areas of the world. First, the evaluation was performed in cell culture against flaviviruses (Japanese encephalitis, yellow fever, and dengue-4), bunyaviruses (Punta Toro, Rift Valley fever, and sandfly fever), alphavirus (Venezuelan equine encephalomyelitis), lentivirus (HIV type 1), and a DNA-containing vaccinia virus. Activity was found only with flaviviruses and bunyaviruses, but even that was observed at concentrations

within 10-fold of those causing toxicity to uninfected control cells. Narciclasine, pancratistatin, isonarciclasine, *trans*- and *cis*-dihydronarciclasine, as well as 7-deoxy analogues of these compounds showed *in vitro* activity against all three flaviviruses, Japanese encephalitis, yellow fever, and dengue-4. All of these compounds are also active against two bunyaviruses, Punta Toro and Rift Valley fever, whereas only 7-deoxy analogues of narciclasine, pancratistatin, and *cis*-dihydronarciclasine exhibit antiviral effects against sandfly fever virus. Among the analogue series, the antiviral activity (expressed as IC₅₀) generally closely parallels toxicity to uninfected cells (expressed as TC₅₀), producing therapeutic indices (TI) values ranging between 3 and 8. Because viruses utilize the cellular protein biosynthetic machinery, it is not unlikely that the observed antiviral effect is solely due to the inhibition of this process by the narciclasine type compounds. Further work is necessary to evaluate whether there is a contribution of a specific antiviral component different from the general inhibition of cellular protein biosynthesis. Notwithstanding, narciclasine, pancratistatin, and 7-deoxypancratistatin show good chemotherapeutic efficacy (>80% survival) in mice infected with low-dose challenge Japanese encephalitis virus. The investigators conclude, "There apparently is a narrow therapeutic margin or poor selectivity. In spite of this, to our knowledge, this provides the most significant example of effective, non-immunomodulatory, antiviral chemotherapy of Japanese encephalitis viral infections in mice."¹⁶⁵

The activity of narciclasine against the Punta Toro virus was further confirmed in an independent study by Sidwell et al., who evaluated 75 compounds in both *in vitro* and *in vivo* models of this infection.¹⁶⁶ Additionally, narciclasine was shown to be active against Rauscher leukemia virus in an *in vitro* model in NIH/3T3 cells; however, the therapeutic window is again very narrow due to its toxicity to host cells at effective antiviral concentrations.¹⁶⁷

7.3. Other Potentially Useful Activities

Ceriotti reported bacteriostatic activity for narciclasine when the isocarbostryril was evaluated against *E. coli* H_pR.⁶⁴ The GI₅₀ value of 8 μg/mL was established. These data are intriguing given the fact that narciclasine does not inhibit protein biosynthesis in *E. coli*,¹¹⁰ nor does it bind to *E. coli* ribosomes, as was established using experiments with [³H]narciclasine (*vide supra*).^{114,115} Pettit et al. also reported marginal antibacterial activity associated with narciclasine derivatives **87** and **92** against *Neisseria gonorrhoeae* (MIC = 32–62 μg/mL).¹⁰⁷ The mechanism of action for these antibacterial effects remains to be elucidated.

Related areas of promise are antifungal activity of narciclasine (*Cryptococcus neoformans*, MIC 8–16 μg/mL)¹⁰⁷ and antiparasitic activity associated with pancratistatin and 7-deoxynarciclasine. More specifically, the latter two isocarbostryrils were reported to inhibit the *in vitro* proliferation of *Encephalitozoon intestinalis*, a microsporidian, which causes intestinal and systemic infections in immunocompromised patients. The IC₅₀ values are 0.18 and 0.2 μM, eight times lower than the corresponding IC₅₀ numbers for toxicity to host cells.¹⁶⁸

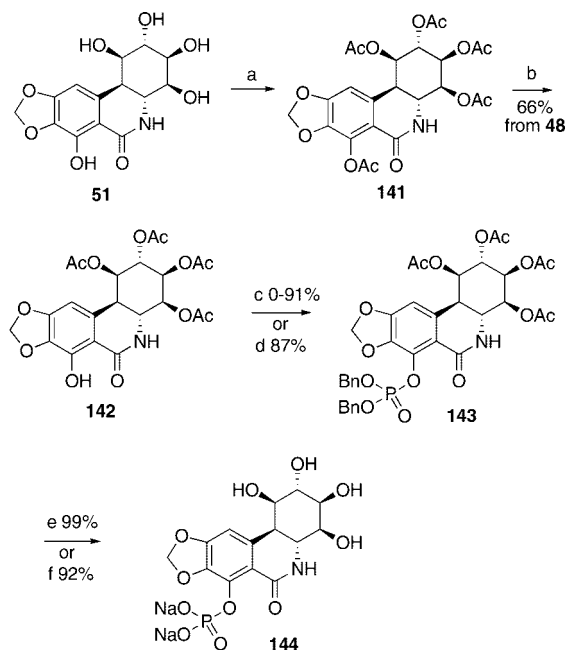
As has been discussed in section 6.3, 2-*O*-β-glucosides of narciclasine (kalbreclasine, **46**) and pancratistatin (pancratistide, **53**) have unusual effects on plant physiology stimulating growth as opposed to the growth-inhibitory action of the nonglycosylated isocarbostryrils. It turns out that the

growth-stimulatory effects of the glucosylated analogues are also observed in animal test models. Kalbreclasine exhibits potent mitogenic action on splenic lymphocytes in healthy adult mice, stimulating their extensive proliferation at concentrations of 20 μg/mL and higher. The effect is comparable to the known mitogen, concanavalin A.⁷⁶

8. Preclinical Studies of Narciclasine and Its Congeners

The preclinical evaluation of narciclasine started with the seminal work by Ceriotti, who demonstrated the isocarbostryril's powerful antimetastatic activity in mice (*vide supra*).⁶⁴ Subsequent studies have been proceeding at a slow pace due to a number of significant challenges. Along with the issue of availability of sufficient quantities of material, which is a general problem for natural product-based drugs, the other important obstacle hampering the clinical development of narciclasine type isocarbostryrils is poor water solubility.¹⁶⁹ A significant effort has been invested in investigating suitable pharmaceutical formulation methods for these agents. For example, methods for entrapment of narciclasine into poly(2-hydroxyethyl)methacrylate matrices for subcutaneous implantation have been reported.^{170,171} Pancratistatin's poor aqueous solubility (53 μg/mL) can be enhanced by the addition of organic solvents, such as DMSO or DMF. Additionally, complex formation with nicotinamide can increase pancratistatin's solubilization to 1 mg/mL. However, the use of organic solvents as formulation components is not desirable, while the anticipated clinical doses of pancratistatin would require unacceptably high doses of nicotinamide.¹⁷² It was also reported that the use of a 40% aqueous solution of hydroxypropyl-β-cyclodextrin (HPCD) solubilizes pancratistatin up to 1.2 mg/mL.¹⁷³ Yet, such solubility is still not sufficient for clinical use. The failure to achieve an acceptable pharmaceutical formulation has led to research efforts aimed at preparation of water-soluble prodrugs. Pettit's group has reported a number of such successful strategies involving the preparations of phosphate derivatives of the natural isocarbostryrils susceptible to the action of human nonspecific phosphatases, which would release the active drug *in vivo*. Pancratistatin-7-*O*-phosphate has been prepared using two different methods for the introduction of the phosphate moiety (Scheme 9).^{174,175} The C ring hydroxyl groups are selectively protected by the preparation of pentaacetate **141** and the subsequent selective hydrolysis of the phenolic acetate by refluxing **141** in water/pyridine. The introduction of the phosphite ester on the phenolic hydroxyl with *i*-Pr₂NP(OBn)₂ and its subsequent oxidation to the corresponding phosphate proved to be irreproducible due to the instability of the phosphorus reagent.¹⁷⁴ An improvement of this method involves the reaction of **142** with dibenzylphosphite and carbon tetrachloride in the presence of *i*-Pr₂NEt and DMAP, which gives dibenzyl phosphate ester **143** directly.¹⁷⁵ Hydrogenolytic removal of the benzyl protection and deacetylation in either sequence (e or f) affords the desired pancratistatin phosphate prodrug as the disodium salt **144**.

Yet, the multistep prodrug preparation starting from the scarcely available natural product prompted Pettit's group to continue their investigation, and a general one-step strategy applicable to the prodrug synthesis for all potent natural isocarbostryrils was found. The method involves reacting the corresponding isocarbostryril with tetrabutylammonium dihydrogenphosphate and dicyclohexylcarbodiimide in pyridine

Scheme 9^a

^a Reagents and conditions: (a) Ac₂O, DMAP, py. (b) H₂O, py, reflux. (c) *i*-Pr₂NP(OBn)₂, tetrazole, CH₂Cl₂; MCPBA, CH₂Cl₂. (d) (BnO)₂P(O)H, DIPEA, DMAP, CCl₄/MeCN. (e) H₂, 10% Pd/C, ethanol; NaOH, H₂O. (f) NaOMe, HOMe; H₂, 10% Pd/C, ethanol; NaOMe, HOMe.

at 80 °C.^{176–178} These conditions result in highly selective formation of 3,4-cyclic phosphates in all cases. The initially formed pyridinium salts are passed through a sodium form of Dowex ion-exchange resin to afford sodium salts of the desired prodrugs. C1-benzoate ester pancratistatin derivative (**105**), whose growth-inhibitory activity exceeds that of pancratistatin by 10–100 times (*vide supra*), was converted to soluble cyclic phosphate derivative (**146**) as well. The structures and yields of the synthesized prodrugs are given in Figure 21.

All of the synthesized phosphate prodrugs were evaluated against human cancer cell lines and found to have slightly weaker growth-inhibitory activities than the corresponding parent compounds as would be expected due to incomplete dephosphorylation of these compounds under the *in vitro* testing conditions.^{176–178} The cytotoxicity profiles among different cell lines are very similar to the corresponding isocarbostryls, demonstrating that the activity results from the dephosphorylated compounds and the dephosphorylation process is expected to be much more efficient *in vivo*. The clinical development of the phosphate prodrugs is anticipated. Intriguing results of preclinical evaluation of pancratistatin prodrugs **144** and **145** were reported by Pettit's group. Compound **144** was investigated for its activity against saphenous vein endothelial cells (SVEC) in culture and a murine colon cancer *in vivo* model using intraperitoneal injection (12.5 mg/kg).¹⁷⁹ In an independent report, cyclic phosphate **145** was tested for its efficacy against human colon adenocarcinoma (DLD-1) in a subcutaneous xenograft model in nude mice using intravenous administration mode (100 mg/kg).¹⁸⁰ Both prodrugs cause an irreversible disruption of cellular cytoskeleton and trigger vascular shutdown in the treated tumors. Disruption of the tumor blood flow results in significant tumor growth inhibition and hemorrhagic necrosis within the tumors. An exciting possibility of the antivasular/antiangiogenesis mechanism for the *in vivo*

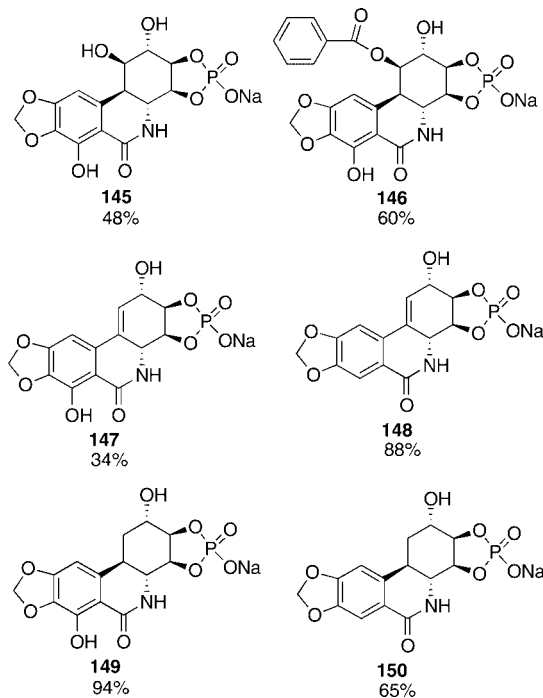


Figure 21. Structures of 3,4-cyclic phosphate prodrugs.

action of both pancratistatin phosphates is emerging and bodes well for the forthcoming clinical testing. Similar experiments with prodrugs **146**–**150** are awaited.

9. Selected Total Synthesis Efforts

With an excellent solution to the water solubility problems of the Amaryllidaceae anticancer isocarbostryls in hand, the issue of supply remains the only major obstacle for completion of preclinical evaluation and advancement of these agents to clinical trials. Pettit and co-workers developed a biotechnological route to pancratistatin, narciclasine, 7-deoxy-narciclasine, and 7-deoxy-*trans*-dihydranarciclasine involving an initial tissue culture cloning of *H. littoralis* bulbs.^{83,104} Although this effort led to the isolation of ca. 15 g of pancratistatin and 12 g of 7-deoxy-narciclasine, these quantities are only sufficient to satisfy an interim demand for these agents. In general, because of the low natural abundance of the Amaryllidaceae anticancer isocarbostryls (Table 2), the isolation from plants cannot be viewed with optimism as a solution to the supply problem. A practical chemical synthesis, on the other hand, could potentially provide a viable alternative. Several dozen completed total syntheses of these compounds are a clear indication of the interest shown by the synthetic community in the challenge presented by these natural products (Table 7). Unfortunately, most of these endeavors focus on showcasing synthetic methodology developed by the investigators, and the majority of the synthetic routes are too long or low yielding to be of practical value. Most of this chemistry has been reviewed;^{16,17,181} therefore, here, the discussion will center on the approaches that upon further optimization can be utilized for the efficient preparation of these medicinally promising agents. The criteria for evaluation of practicality of synthetic efforts have been discussed in an excellent review by Hudlicky,¹⁸² and they are used in our critical analysis of the relevant synthetic pathways.

Of the many strategies described in the literature, the chemoenzymatic approach by Hudlicky's group and radical

Table 7. Reported Total Syntheses of the Amaryllidaceae Isocarbostryils

isocarbostryril	research group	publication year	no. of steps ^a	overall yield (%) ^a	refs
(+)-narciclasine	Rigby	1997	23	0.2	183, 184
(+)-narciclasine	Hudlicky	1999	9	0.6	146, 185
(+)-narciclasine	Keck	1999	14	16	162
(+)-narciclasine	Yan	2002	13	17	186
(±)-pancratistatin	Danishefsky	1989	27	0.16	187
(+)-pancratistatin	Hudlicky	1995	14	2	188, 189
(+)-pancratistatin	Trost	1995	19	8	190
(+)-pancratistatin	Haseltine	1997	24	0.97	191
(+)-pancratistatin	Magnus	1998	22	1.2	192, 193
(+)-pancratistatin	Rigby	2000	23	0.35	184
(+)-pancratistatin	Pettit	2001	10	3.6	109 ^b
(±)-pancratistatin	Kim	2002	21	4	194, 195
(+)-pancratistatin	Li	2006	13	9	196
(±)-7-deoxynarciclasine	Ohta, Kimoto	1975	20	1.5	197, 198, 199
(+)-7-deoxynarciclasine	Paulsen	1982	12	4	200, 201
(±)-7-deoxynarciclasine	Schubert	1987	18	7.2	202 ^c
(+)-7-deoxynarciclasine	Ogawa	1991	23	0.04	203, 204
(+)-7-deoxynarciclasine	Hudlicky	1992	8	12	205, 206
(±)-7-deoxynarciclasine	Martin	1993	11	4	207
<i>ent</i> -7-deoxynarciclasine	Keck	1996	14	11	161, 162
(+)-7-deoxynarciclasine	Keck	1999	11	27	162
(+)-7-deoxynarciclasine	Yan	2002	15	11	208
(±)-7-deoxynarciclasine	Padwa	2006	15	10	209, 210
(+)-7-deoxypancratistatin	Paulsen	1982	9	6.5	200, 201
(+)-7-deoxypancratistatin	Keck	1995	22	4	211, 212
(+)-7-deoxypancratistatin	Hudlicky	1995	12	2.6	213
(+)-7-deoxypancratistatin	Hudlicky	1995	10	3	189, 213
(+)-7-deoxypancratistatin	Chida	1996	29	0.03	214
(+)-7-deoxypancratistatin	Keck	1998	15	12	215
<i>ent</i> -(-)-7-deoxypancratistatin	Hudlicky	1999	14	1	163
(+)-7-deoxypancratistatin	Plumet	2000	21	3	216
(+)-7-deoxypancratistatin	Madsen	2006	13	1.4	217
(+)-7-deoxypancratistatin	Madsen	2006	15	4.3	217
(±)-7-deoxypancratistatin	Padwa	2006	23	3	210, 218
(±)- <i>trans</i> -dihydronarciclasine	Cho	2007	15	11	219
(±)-7-deoxy- <i>trans</i> -dihydronarciclasine	Tsuda	1978	16	1.1	220
(+)-7-deoxy- <i>trans</i> -dihydronarciclasine	Chida	1996	24	0.18	214
(+)-7-deoxy- <i>trans</i> -dihydronarciclasine	Iwabuchi	2005	24	1.4	221

^a Number of steps and overall yield from commercially available starting material for the longest linear sequence. One-pot procedures are counted as one step. If the utilized starting material was not commercially available but had been reported previously by other investigators, the number of steps and yields was taken from the cited sources. ^b Relay synthesis from (+)-narciclasine. ^c Improvement of the synthesis by Ohta and Kimoto (ref 201).

cyclization chemistry developed by Keck's group are quite general and can potentially lead to efficient preparations of pancratistatin, narciclasine, 7-deoxynarciclasine, and 7-deoxypancratistatin. Additionally, syntheses of (+)-narciclasine and (+)-pancratistatin reported by Yan's and Li's groups, respectively, are short and high yielding. Unfortunately, efficient synthetic routes to *trans*-dihydronarciclasine and 7-deoxy-*trans*-dihydronarciclasine remain elusive. The only synthesis of *trans*-dihydronarciclasine was recently reported by Cho and co-workers.²¹⁹ Although relatively short and high yielding, the synthesis leads to racemic material, and therefore, rather low-yielding hydrogenation of (+)-narciclasine (Scheme 1) is the only preparation of the enantiomerically pure *trans*-dihydronarciclasine at the time of this writing.

9.1. Chemoenzymatic Strategy to (+)-Pancratistatin, (+)-7-Deoxypancratistatin, and (+)-7-Deoxynarciclasine by Hudlicky and Co-workers

The chemoenzymatic approach to the Amaryllidaceae anticancer isocarbostryils by Hudlicky and co-workers is based on the oxidation of bromobenzene by a bacterial dioxygenase enzyme of *Pseudomonas putida* in the whole cell laboratory fermentation apparatus.²²² Although relatively

expensive, diol **151** (Figure 22) is now available commercially (201 USD/5 g, Aldrich). Regioselective aziridination (A) and aziridine ring opening with an appropriately functionalized cuprate reagent provides B, possessing four of the six stereocenters in pancratistatin and 7-deoxypancratistatin with the correct relative and absolute stereochemistries. Lactamization and *trans*-diol installation through olefin epoxidation and epoxide *trans*-diaxial ring opening gives the targets **51** and **52**. Alternatively, Diels–Alder reaction of appropriately protected **151** with an acyl nitroso compound followed by N–O bond cleavage and *N*-acylation to introduce the aromatic residue give C. The intramolecular Heck reaction then affords the target **45**.

The strategy was implemented in the synthesis of pancratistatin as follows.^{188,189} Diol **151** is protected as acetonide and then treated with (*N*-tosylimino)phenyliodine and Cu(acac)₂ catalyst to generate aziridine **152** in a highly regioselective manner (Scheme 10). Radical debromination leads to **153**, which is reacted with the highly functionalized arylcuprate **154** in an S_N2 fashion (as opposed to S_N2') affording the coupled product **155** in good yield. Such rapid build-up of complexity and installation of four key stereocenters present in the target molecule make this sequence extremely efficient. However, the subsequent troublesome formation of atropoisomers and the difficulties posed by the stable tosylamide and dimethylamide functionalities greatly

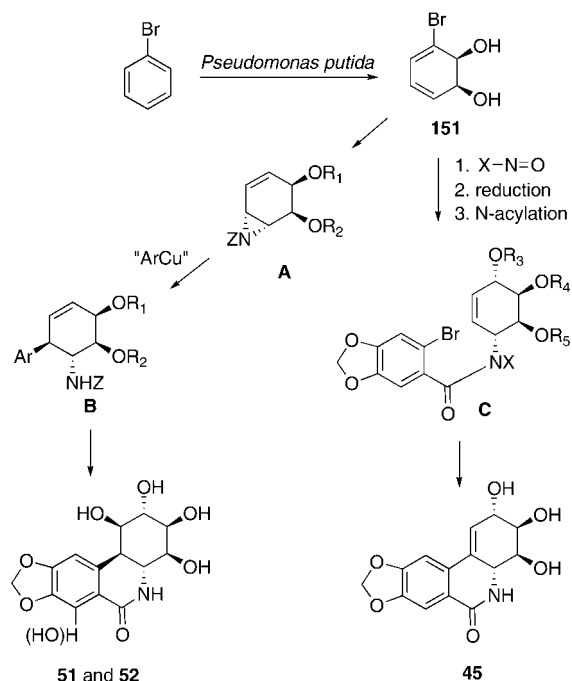


Figure 22. Chemoenzymatic strategy to (+)-pancratistatin, (+)-7-deoxypancratistatin, and (+)-7-deoxynarciclasine.

diminish the practicality of the overall synthetic sequence. Reductive removal of tosylamide is facilitated by the introduction of the Boc moiety to give **156**, which exists as a mixture of atropoisomers that undergo reductive detosylation at different rates and even with different outcomes. Thus, the major detosylated compound **157 β** retains the TBS ether group after treatment with Na/anthracene, while the minor **157 α** is obtained with the free phenolic hydroxyl. Therefore, **157 β** is further desilylated to converge on the **157 α** atropoisomer. Removal of the dimethylamide group is achieved by reduction of the latter to the aldehyde moiety with sodium bis(methoxyethoxy)aluminum hydride and subsequent oxidation and methylation with diazomethane to obtain ester **159**. Unmasking of the allylic hydroxyl in **159** allows for β -selective epoxidation to give **160**. Finally, reflux in aqueous sodium benzoate solution accomplishes a number of transformations, namely, epoxide ring opening, cleavage of the Boc protection, lactamization, and debenzoylation of the phenolic hydroxyl, giving the target molecule directly in 51% yield.

This short 14-step synthesis proceeds in a rather low 2% overall yield due to the impediments mentioned above. Further optimization is undoubtedly required to reduce this promising strategy to a practical preparation of (+)-pancratistatin.

The highly functionalized arylcuprate **154** is prepared from piperonal by utilizing a clever directed metalation sequence. Both Hudlicky's¹⁸⁹ and later Keck's¹⁶² groups have shown this efficient way of construction of such regiochemically complex benzene derivatives, which in principle can be generally applicable to any pancratistatin or narciclasine syntheses (Scheme 11).

The aldehyde group of piperonal (**161**) is converted to dimethylamide **162** by a one-step procedure involving treatment of the former with MnO₂ in the presence of Me₂NH. First *ortho*-lithiation directed by the amide functionality is followed by the treatment of the aryl lithium intermediate with trimethylborate and oxidation of the

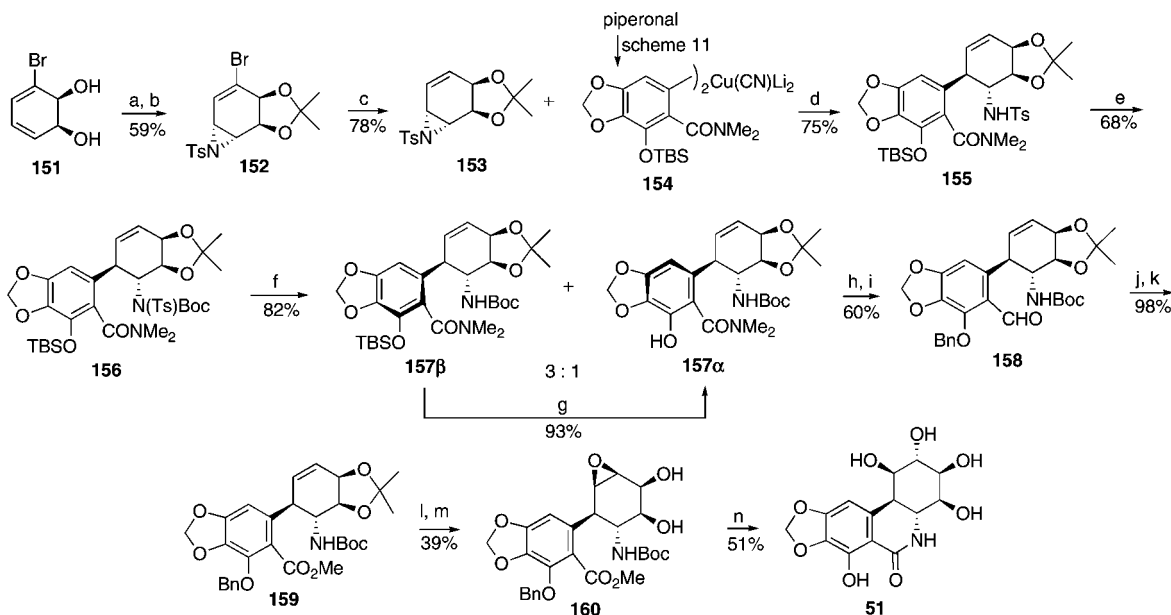
resulting boronate with hydrogen peroxide to yield phenol **163**. The latter is protected as TBS ether to afford tetrasubstituted benzene **164**. Second lithiation, now at the other *ortho*-position of the dimethylamide functionality, affords aryl iodide **165** after the aryl lithium is quenched with iodine or results in the formation of higher order cyanocuprate **154** when the aryl lithium is treated with copper(I) cyanide. Lastly, Keck and co-workers showed that the amide can be converted to the corresponding ester upon treatment with trimethyloxonium tetrafluoroborate.¹⁶² The freed under the reaction conditions phenolic hydroxyl can be further protected with a group capable of modulating the electronic properties of the benzene ring, for example, tosyl ester **166** optimized for Sonogashira coupling in their synthesis of (+)-narciclasine (vide infra).

The second generation synthesis of (+)-7-deoxypancratistatin by Hudlicky's group was modified to avoid problems encountered in their route toward pancratistatin, namely, abstaining from the use of dialkylamide and sulfonamide functionalities.¹⁸⁹ Thus, instead of *N*-tosyl aziridine **152**, the investigators prepared *N*-carbomethoxy aziridine **167** (Scheme 12). Radical debromination followed by the S_N2 addition of cuprate **169** gives carbamate **170**. The latter is deprotected and subjected to allylic hydroxyl-directed β -epoxidation to give **171**, which undergoes smooth *trans*-diaxial epoxide opening affording carbamate **172**. Finally, exhaustive acetylation, regioselective Bischler-Napieralski cyclization, and deacetylation result in the target natural isocarbostryl **52**.

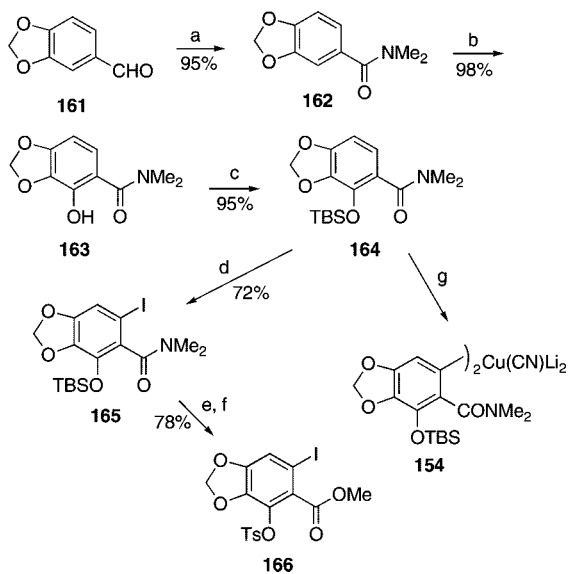
This very short (10 steps from diol **151** or 11 steps from bromobenzene) synthesis unfortunately proceeds in rather low overall yield of 3%. The key steps that undermine the yield are aziridination, radical debromination, and aziridine ring opening. Given a large number of aziridination protocols available in the literature and the flexibility of cuprate chemistry, it is highly possible that these impediments can be overcome to provide a scalable synthetic route to this natural product.

The chemoenzymatic strategy allows for a very efficient synthesis of (+)-7-deoxynarciclasine (Scheme 13).^{205,206} Diels–Alder reaction of diene **173** with in situ-generated BnOCON=O gives bicyclic oxazine **174**, in a highly stereo- and regioselective manner. Na(Hg)-promoted reductive cleavage of first C–Br and then N–O bonds gives *syn*-aminoalcohol, which is silylated to afford carbamate **175** in good yield over three steps. *N*-Acylation of **175** with acid chloride **176** leads to the coupled product **177**, which undergoes a palladium-catalyzed Heck type ring closure providing a modest yield of phenanthridone **178**. Finally, transfer hydrogenolysis of the Cbz group and removal of the acetonide protection with CF₃CO₂H give the target isocarbostryl **45**.

This pathway constitutes the shortest synthesis of (+)-7-deoxynarciclasine (eight steps from diol **151**, nine steps from bromobenzene) reported to date in excellent (12%) overall yield, and it should be well-suited for a large scale preparation of this anticancer natural product. The weakest step is the cyclization of **177** to **178**. This unconventional Heck type transformation involving a formal *anti* β -hydride elimination is reasonably well-precedented but is still poorly understood.^{223,224} Further study of its mechanism is warranted, and it should directly impact the efficiency of this and many other syntheses.

Scheme 10^a

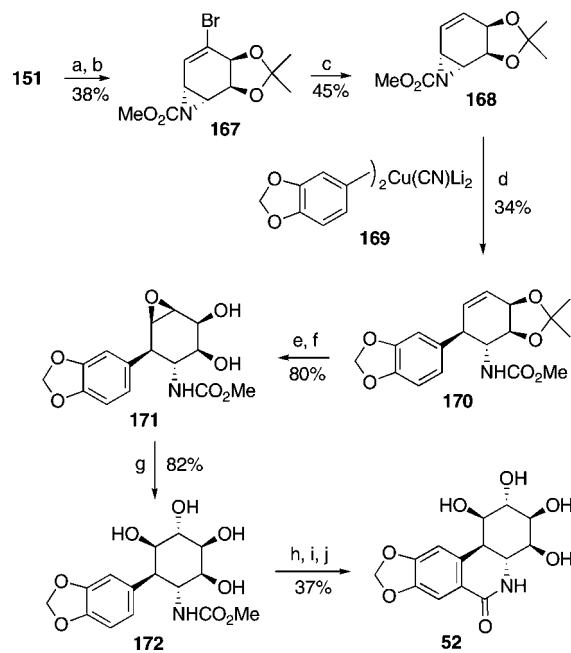
^a Reagents and conditions: (a) $\text{Me}_2\text{C}(\text{OMe})_2$, *p*-TsOH, CH_2Cl_2 . (b) $\text{PhI}=\text{NTs}$, $\text{Cu}(\text{acac})_2$, MeCN. (c) Bu_3SnH , AIBN, THF. (d) THF, -78°C to room temperature. (e) *s*-BuLi, THF, $(\text{Boc})_2\text{O}$. (f) Na/anthracene, DME, -78°C . (g) TBAF, THF. (h) $\text{NaAl}(\text{MeOCH}_2\text{CH}_2)_2\text{H}_2$, morpholine, -45°C , THF. (i) BnBr, K_2CO_3 , DMF. (j) NaClO_2 , KH_2PO_4 , 2-methyl-2-butene, *t*-BuOH, H_2O . (k) CH_2N_2 . (l) AcOH, THF, H_2O , 60°C . (m) *t*-BuOOH, $\text{VO}(\text{acac})_2$, PhH, 60°C . (n) BzONa (cat.), H_2O , 100°C .

Scheme 11^a

^a Reagents and conditions: (a) MnO_2 , NaCN, Me_2NH , *i*-PrOH. (b) *n*-BuLi, TMEDA, -104°C ; $\text{B}(\text{OMe})_3$, THF; AcOH, H_2O_2 . (c) TBSCl, imidazole. (d) *n*-BuLi, TMEDA, -104°C , Et_2O , I_2 . (e) Me_3OBF_4 , Na_2HPO_4 , MeCN. (f) TSCl, py. (g) (i) *s*-BuLi, TMEDA, THF, -90°C ; (ii) CuCN, -90 to -20°C .

9.2. Radical Cyclization Strategies to (+)-Narciclasine, (+)-7-Deoxynarciclasine, and (+)-7-Deoxypancratistatin by Keck and Co-workers

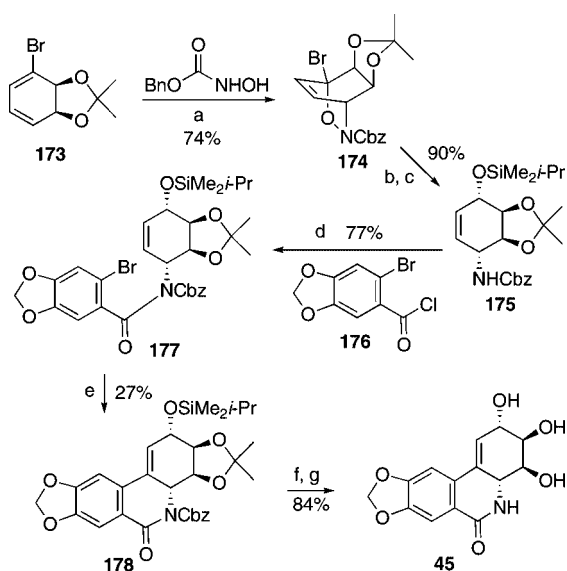
Keck and co-workers have explored a number of radical cascade cyclizations for the construction of the phenanthridone skeleton associated with the Amarylidiaceae anticancer constituents. These efforts resulted in efficient syntheses of (+)-narciclasine, (+)-7-deoxynarciclasine, and (+)-7-deoxy-

Scheme 12^a

^a Reagents and conditions: (a) $\text{Me}_2\text{C}(\text{OMe})_2$, *p*-TsOH, CH_2Cl_2 . (b) *p*- $\text{O}_2\text{NPhSO}_3\text{NHCO}_2\text{Me}$, K_2CO_3 , CH_2Cl_2 . (c) Bu_3SnH , AIBN, THF. (d) $\text{BF}_3 \cdot \text{Et}_2\text{O}$, THF, -78 to -30°C . (e) AcOH, THF, H_2O , 65°C . (f) *t*-BuOOH, $\text{VO}(\text{acac})_2$, PhH, 60°C . (g) BzONa (cat.), H_2O , 100°C . (h) Ac_2O , DMAP, py. (i) Tf_2O , DMAP, CH_2Cl_2 , 5°C . (j) MeONa, MeOH.

pancratistatin. Their strategy for the syntheses of (+)-narciclasine and (+)-7-deoxynarciclasine is shown in Figure 23.

Regioselective addition of a suitable radical to the alkyne moiety of A was expected to give an intermediate vinyl radical B, which would undergo cyclization onto the C=N bond of the pendant oxime functionality. The strategy was implemented as follows.¹⁶²

Scheme 13^a

^a Reagents and conditions: (a) Bu_4NIO_4 , CH_2Cl_2 . (b) $\text{Al}(\text{Hg})$, THF. (c) $i\text{-PrMe}_2\text{SiCl}$, imidazole, CH_2Cl_2 . (d) BuLi , THF, -78°C . (e) $\text{Pd}(\text{OAc})_2$, $\text{Ti}(\text{OAc})_3$, DIPHOS, anisole. (f) Pd/C , cyclohexene, EtOH. (g) $\text{CF}_3\text{CO}_2\text{H}$, 0°C .

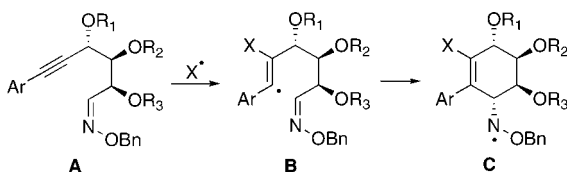
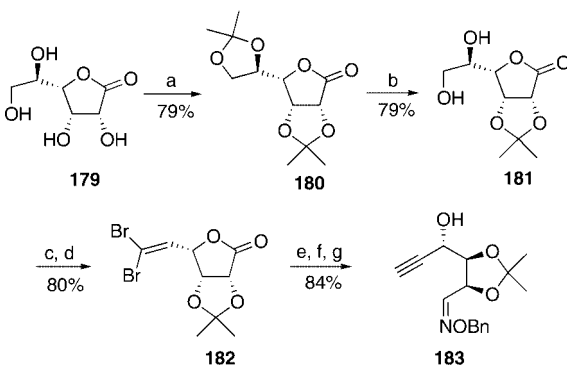


Figure 23. Radical cyclization strategy to (+)-narciclasine and (+)-7-deoxynarciclasine.

Scheme 14^a

^a Reagents and conditions: (a) $\text{Me}_2\text{C}(\text{OMe})_2$, $p\text{-TsOH}$. (b) AcOH , H_2O . (c) NaIO_4 , CH_2Cl_2 . (d) CBr_4 , PPh_3 , NEt_3 . (e) $L\text{-Selectride}$, Et_2O , -78°C . (f) $\text{HCl}\cdot\text{H}_2\text{NOBn}$, py. (g) $n\text{-BuLi}$, Et_2O , -90°C .

Readily available D-gulonolactone (**179**, 79 USD/25 g, Aldrich) is converted to diacetone derivative **180**, in which the C5, C6 diol is selectively unmasked upon treatment with mild acid to give **181** (Scheme 14). Periodate cleavage of the diol functionality followed by Corey-Fuchs reaction result in dibromoalkene **182**. The lactone is further reduced to the corresponding lactol, which is converted to *O*-benzyl oxime and then treated with BuLi to form terminal alkyne **183** in excellent yield over the three-step sequence.

Alkyne **183** serves as a common precursor for the synthesis of both iscarbostyrils. To reach narciclasine, the investigators performed Sonogashira coupling of terminal alkyne in **183** with aryl iodide **166** to give **184**, and this sets the stage

for the key radical cyclization reaction (Scheme 15). Phenylthiyl radical adds high regioselectivity to the triple bond of **184** (presumably because of the benzylic stabilization), and the resulting intermediate vinyl radical undergoes ring closure with the *O*-benzyl oxime moiety acting as the final radical acceptor. Chemoselective reduction of the tosylate in **185** and re-protection of the phenol as methyl ether are followed by the treatment with Me_3Al to induce cyclization to a fully assembled narciclasine skeleton in **187**. Finally, SmI_2 reduction results in *N*-*O* bond cleavage and the removal of the PhS group with the concomitant loss of the phenolic methyl to give narciclasine acetonide. The latter is converted to narciclasine (**44**) by the treatment with trifluoroacetic acid.

Overall, the synthesis proceeds in 14 steps from gulonolactone (**179**) and an excellent 16% yield. Although the phenolic protection strategies in the form of tosylate ester and methyl ether are optimized for the palladium-catalyzed coupling of **183** and **166**, radical cyclization of **184**, and the cascade reduction processes of **187**, it should be possible to considerably shorten the synthesis by further investigating and possibly limiting the phenolic protection/deprotection manipulations.

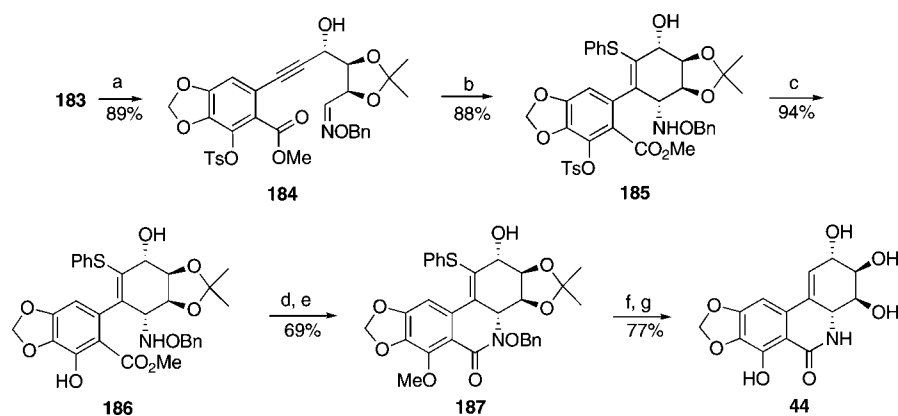
Sonogashira coupling of **183** with aryl iodide **188** gives **189** and sets the stage for the radical cyclization leading to (+)-7-deoxynarciclasine (Scheme 16). In the event, phenylthiyl radical-promoted cyclization proceeds as cleanly and regioselectively as the corresponding process used in the construction of the narciclasine skeleton. Reduction of the cyclized product **190** with SmI_2 leads to *N*-*O* and *C*-*S* bonds cleavage and lactamization. Finally, acetonide removal furnishes the target compound **45**.

Overall, the synthesis proceeds in just 11 steps from gulonolactone and excellent 27% yield. It is noteworthy that C2-hydroxyl (narciclasine numbering) needs no protection in the second half of the synthesis, enhancing the efficiency of the synthetic route.

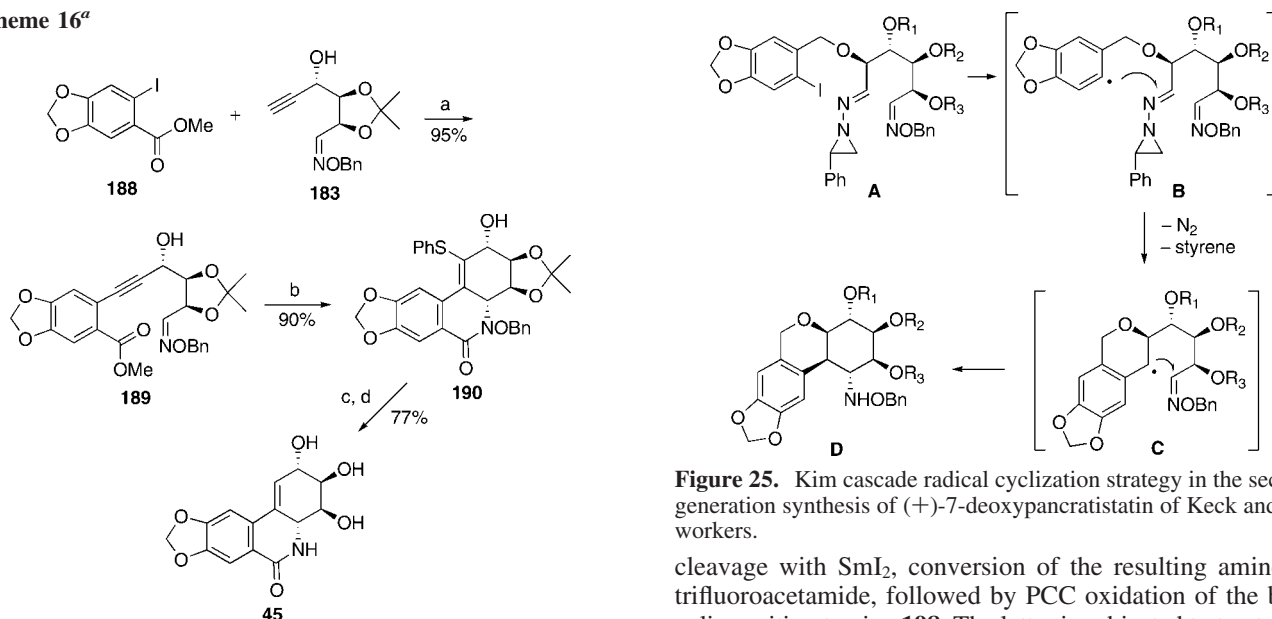
Keck and co-workers have also applied such radical cyclization approaches to the synthesis of (+)-7-deoxypancratistatin. Their first generation synthesis is based on the cyclization of the benzylic radical B onto a pendant oxime moiety giving lactol structure C, which is further converted to the target iscarbostyril by rearrangement to the corresponding lactam (Figure 24).

Unfortunately, such a strategy resulted in a rather long 22 step synthetic sequence.^{211,212} A significantly improved second generation synthesis utilizes Kim cascade radical cyclization process involving the use of an aziridine imine moiety (A, Figure 25). The intramolecular addition of aryl radical to the *C*=*N* bond of the aziridine imine (B) leads to the loss of nitrogen and styrene regenerating a carbon-based benzylic radical C, which undergoes a second cyclization onto the *C*=*N* bond of the oxime functionality completing the construction of the desired multicyclic skeleton D.²¹⁵

Such second generation synthesis was accomplished as follows (Scheme 17). Gulonolactone-derived diol **181** is selectively silylated at the primary hydroxyl and then subjected to benzyl ether formation under acidic conditions to give **193**. Reduction of the lactone to the corresponding lactol moiety, followed by oxime formation, results in **194**. Silylation of the secondary hydroxyl in **194** and selective cleavage of the primary silyl ether afford alcohol **195**. Oxidation of the latter to the corresponding aldehyde is followed by its conversion to the desired *N*-aziridinylimine

Scheme 15^a

^a Reagents and conditions: (a) Compound **166**, Pd(OAc)₂, CuI, NEt₃, PPh₃, THF. (b) PhSH, hν, PhMe, 27 °C. (c) SmI₂, THF, H₂O, 0 °C. (d) MeI, K₂CO₃, DMF. (e) Me₃Al, THF. (f) SmI₂, MeOH, THF, 0 °C. (g) TFA, 0 °C.

Scheme 16^a

^a Reagents and conditions: (a) Pd(OAc)₂, CuI, NEt₃, PPh₃, THF. (b) PhSH, hν, PhMe, 27 °C. (c) SmI₂, THF. (d) TFA, 0 °C.

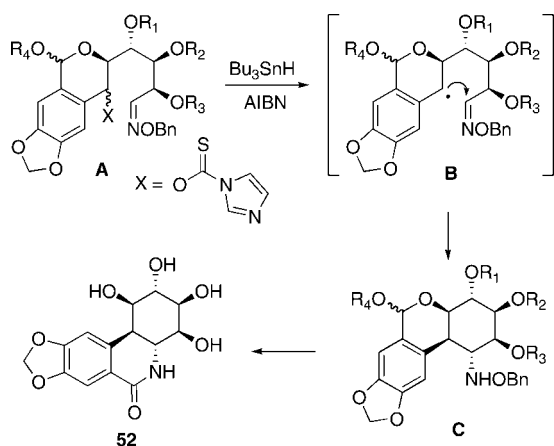


Figure 24. Radical cyclization strategy in the first generation synthesis of (+)-7-deoxypancratistatin of Keck and co-workers.

196. The key radical cascade cyclization event proceeds cleanly with the use of Ph₃SnH to give **197** as a single diastereomer in excellent yield for such a complex transformation. The completion of the synthesis involves N–O bond

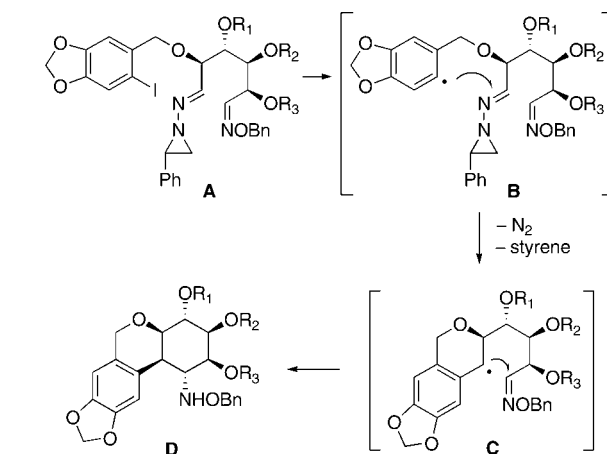


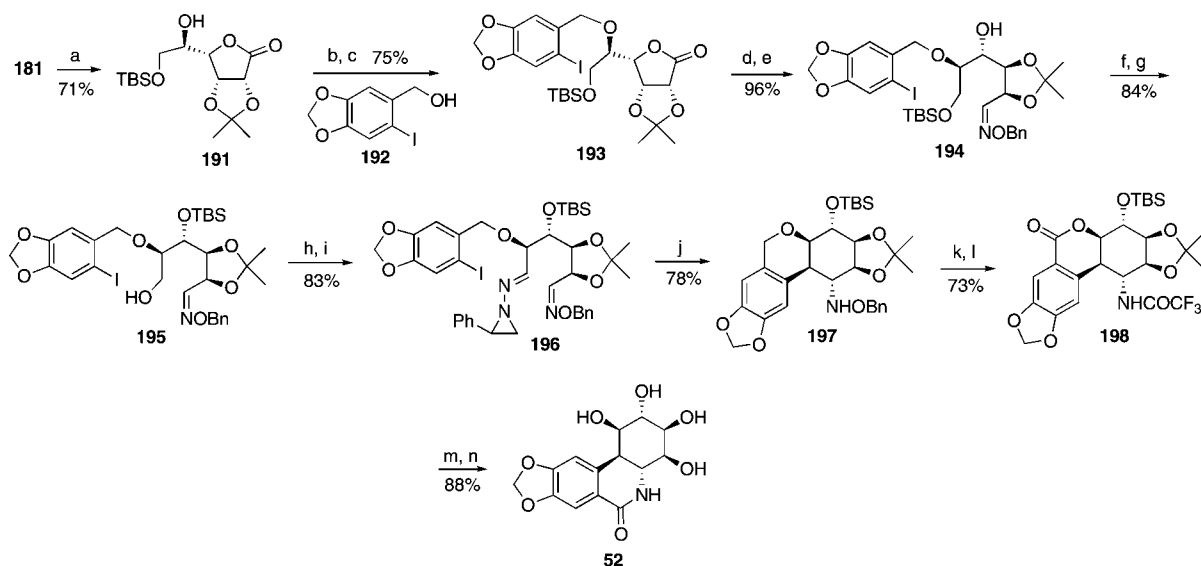
Figure 25. Kim cascade radical cyclization strategy in the second generation synthesis of (+)-7-deoxypancratistatin of Keck and co-workers.

cleavage with SmI₂, conversion of the resulting amine to trifluoroacetamide, followed by PCC oxidation of the benzylic position to give **198**. The latter is subjected to treatment with BF₃·Et₂O in CH₂Cl₂, resulting in removal of the acetonide and silyl ether protecting groups. Lactone to lactam rearrangement promoted by potassium carbonate in methanol likely proceeds via the intermediacy of methyl ester and gives the target isocabostyryl **52** in excellent yield.

Overall, the synthesis requires 15 linear steps from D-gulonolactone and proceeds in very good 12% overall yield. It should be noted that the syntheses of (+)-7-deoxynarciclasine and (+)-7-deoxypancratistatin utilizing the radical cyclization strategies are slightly longer but higher yielding than the chemoenzymatic synthetic pathways to these natural products discussed above. Both of these approaches are very general, permitting access to a number of the Amaryllidaceae anticancer isocabostyryls, and with further optimization, they can be well-suited for practical preparation of these agents for clinical evaluation.

9.3. Synthesis of (+)-Narciclasine by Yan and Co-workers

Yan and co-workers described an efficient synthesis of (+)-narciclasine¹⁸⁶ based on an intramolecular Lewis acid-promoted arene-epoxide coupling following an established precedent from the work of Hudlicky et al.^{146,225} on the synthesis of 10b-*epi*-7-deoxypancratistatin (Figure 26).

Scheme 17^a

^a Reagents and conditions: (a) TBSCl, imidazole. (b) Compound **192**, NaH, Cl₃CCN, 0 °C. (c) TfOH, THF, 0 °C. (d) L-Selectride, CH₂Cl₂, -78 °C. (e) HCl·H₂NOBn, py. (f) TBSOTf, 2,6-lutidine, CH₂Cl₂, 0 °C. (g) HF·py, THF. (h) TPAP, NMO, 4 Å MS. (i) 1-Amino-2-phenylaziridine, EtOH, 0 °C. (j) Ph₃SnH, AIBN, PhH, 78 °C. (k) SmI₂, THF, then TFAA. (l) PCC, CH₂Cl₂, 55 °C. (m) BF₃·Et₂O, CH₂Cl₂. (n) K₂CO₃, MeOH.

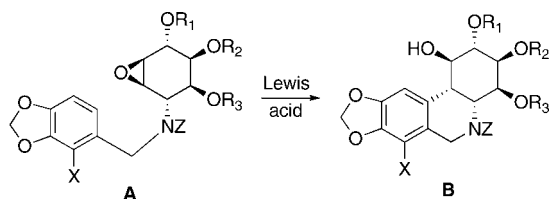


Figure 26. Strategy to (+)-narciclasine based on intramolecular arene-epoxide ring opening.

The preparation of aminoconduritol **202**, possessing all four stereocenters in the structure of narciclasine with the correct relative and absolute stereochemistries, is accomplished by the asymmetric cycloaddition of the chloronitroso compound **200** with diene **201**, followed by the reductive N–O cleavage with Al–Hg (Scheme 18). Compound **200** is derived from ketopinic acid (**199**, 65 USD/1 g, Aldrich). Although the starting material is somewhat expensive, ketoester **203** is isolated by simple extraction of the aqueous reaction mixture in 93% yield and can be recycled. Protection of the hydroxyl and amino groups in aminoconduritol **202** (>99% ee) as TBS ether and sulfonamide, respectively, gives **204**. The introduction of the required epoxide at the β -face of olefin **204** is achieved through nonregioselective bromohydrin formation, possibly via the intermediacy of a bromonium ion at the convex α -face of the molecule followed by the unselective attack by water. Both bromohydrins, **205** and **206**, lead to the same β -epoxide **207** when their 1:1 mixture is treated with potassium carbonate in MeCN. In situ addition of bromide **208** to the reaction mixture results in concomitant *N*-piperonylation giving **209** in excellent overall yield for this complex transformation. The crucial arene-epoxide cyclization is cleanly promoted by tin tetrachloride affording a quantitative yield of **210** after acetylation of the cyclized material. The completion of the synthesis involves nitrogen reprotection and oxidation of the benzylic position to form lactam **212**. This is followed by the *syn*-elimination of the acetoxy group with DBU in hot benzene and complete deprotection to give the target molecule.

The synthesis is very efficient requiring 13 steps to reach the target compound in an excellent 17% overall yield.

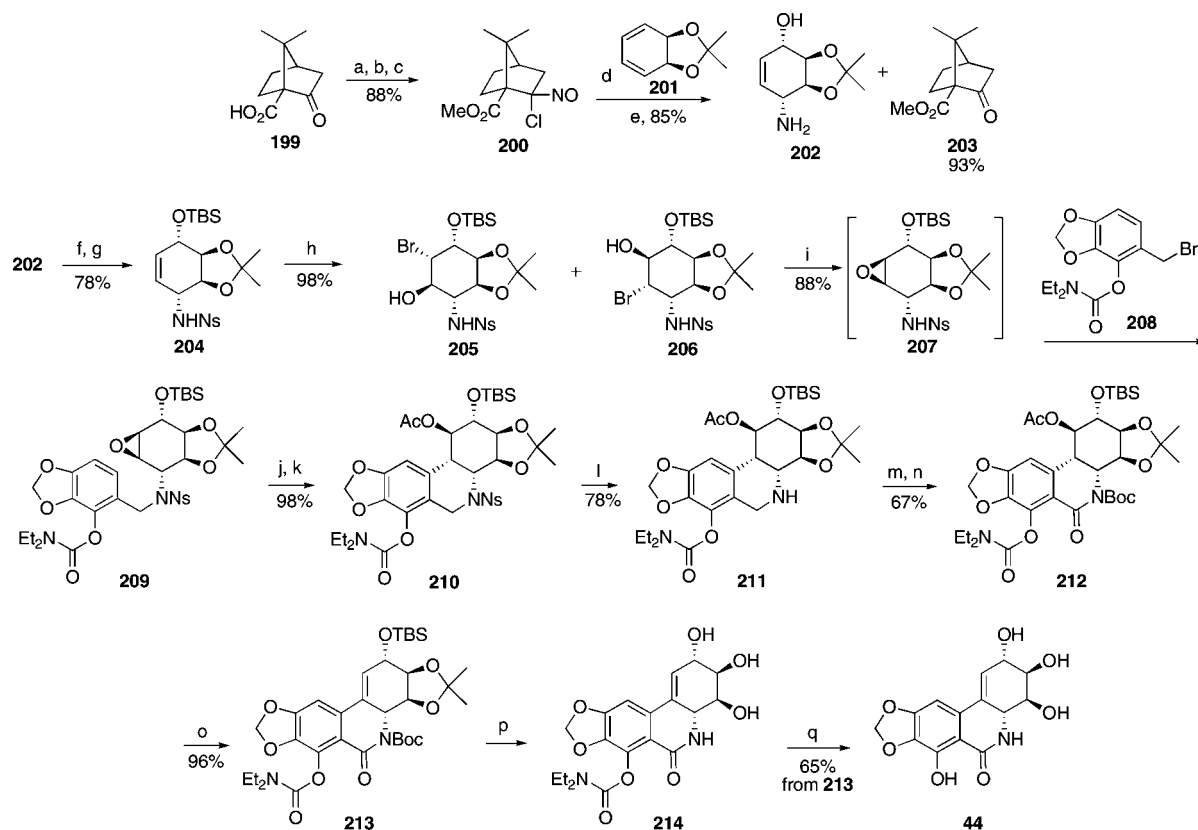
Although the starting material, ketopinic acid (**199**), is not inexpensive, the procedure involving its recycling mitigates the cost problem. Further improvements can be made by reducing the number of protecting groups as well as protection/deprotection steps (e.g., **210** \rightarrow **212**).

9.4. Synthesis of (+)-Pancratistatin by Li and Co-workers

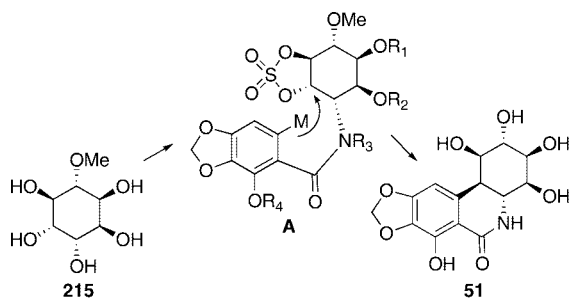
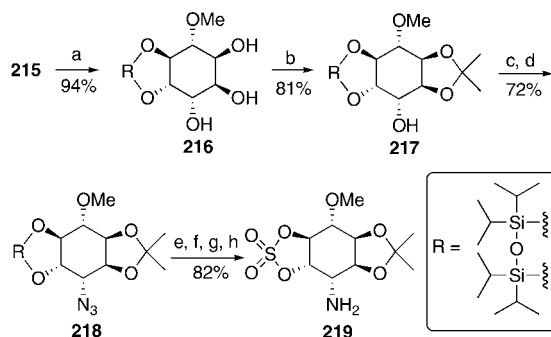
Li and co-workers have developed an efficient route to (+)-pancratistatin utilizing a strategy based on intramolecular cyclic sulfate ring opening with an arylmetal nucleophile (A, Figure 27).¹⁹⁶ Pinitol (**215**, 34 USD/0.5 g, Aldrich) represents somewhat expensive, but attractive starting material as it already incorporates four stereogenic centers found in (+)-pancratistatin.

Selective protection of the *trans* (**215** \rightarrow **216**) and *cis* (**216** \rightarrow **217**) diols leaves C6-hydroxyl in compound **217** available for the replacement with a nitrogen functionality, which is present in pancratistatin at this position with the same stereochemistry (Scheme 19). This is accomplished by Mitsunobu inversion with methylsulfonic acid and the subsequent substitution with azide ion giving azide **218** with the overall retention of configuration. Removal of the silyl protection, introduction of the cyclic sulfate at the unmasked *trans*-diol moiety, and Staudinger azide reduction yield cyclic sulfate-containing amine **219**, ready for coupling with an aromatic residue.

The aromatic acid chloride coupling partner is prepared from bromide **220** by removal of the PMB protecting group and treatment of the resulting phenol with MgBr₂·OEt₂ and phosgene in ether (Scheme 20). This procedure gives the presumed acid chloride complex **221**, which is reacted in situ with amine **219** affording the coupled product **222**. MOM protection of the NH and OH groups gives **223** and sets the stage for the crucial ring forming intramolecular cyclic sulfate ring opening reaction. The investigators found that treatment of bromide **223** with *t*-BuLi, followed by the addition of anhydrous CeCl₃, leads to the desired transformation via the presumed arylcerium intermediate. Finally,

Scheme 18^a

^a Reagents and conditions: (a) SOCl₂, MeOH. (b) HCl·NH₂OH, MeOH, AcONa. (c) *t*-BuOCl, CH₂Cl₂. (d) CH₂Cl₂, 0 °C, 12 h. (e) Al-Hg, H₂O/MeCN. (f) *p*-O₂NPhSO₂Cl, MeCN, NEt₃. (g) TBSCl, DBU. (h) NBS, H₂O/acetone. (i) MeCN, K₂CO₃, 60 °C, then **208**. (j) 20 mol % SnCl₄, CH₂Cl₂. (k) Ac₂O, K₂CO₃. (l) HSCH₂CO₂H, LiOH, DMF. (m) Boc₂O, MeCN. (n) RuCl₃, NaIO₄, H₂O. (o) DBU, PhH, 70 °C. (p) HCO₂H, THF, 60 °C. (q) LiAlH₄, THF.

Scheme 19^a

^a Reagents and conditions: (a) TIPDSCl₂, imidazole, DMAP. (b) Me₂C(OMe)₂, *p*-TsOH. (c) PPh₃, DEAD, MeSO₃H, CH₂Cl₂. (d) NaN₃, DMF, 60 °C. (e) TBAF, THF. (f) SOCl₂, Et₃N, CH₂Cl₂. (g) NaIO₄, RuCl₃, aqueous MeCN. (h) PPh₃, aqueous THF.

Figure 27. Strategy to (+)-pancratistatin based on intramolecular ring opening of a cyclic sulfate moiety.

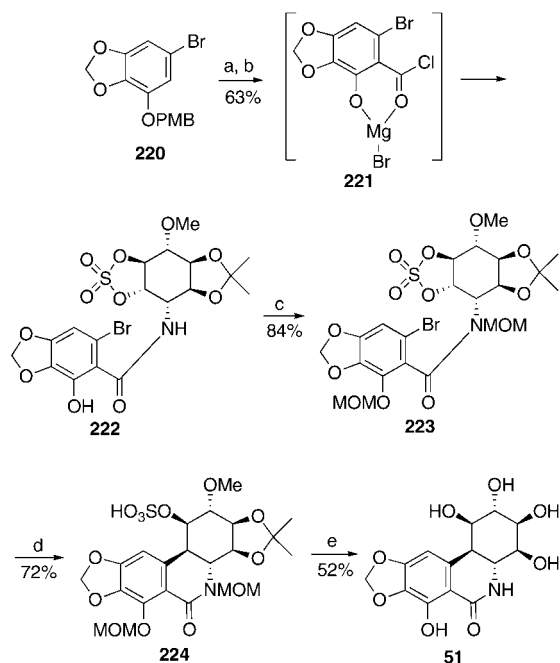
complete deprotection is accomplished by treating **224** with BBr₃ in CH₂Cl₂, followed by the addition of MeOH under careful temperature control to avoid the loss of the methylenedioxy unit.

This synthesis represents the shortest (13 steps from pinitol) and the most high-yielding (9% overall) route from commercially available starting material to (+)-pancratistatin reported so far. At the time of this writing, the results have been disclosed in a communication format only, and the full article, detailing the experimental nuances is eagerly awaited.

10. Concluding Remarks

The use of Amaryllidaceae plants, particularly of the genus *Narcissus*, for cancer management beginning with Hippocrates in the fourth century B.C., later by medical practitioners of the middle ages throughout the world, and currently through ongoing research efforts has advanced our knowledge to the point of having identified the specific small

molecule constituents of these plants that possess potent anticancer properties. As most researchers now believe, these constituents include isoquinolines narciclasine, pancratistatin, *trans*-dihydronarciclasine, and their corresponding 7-deoxy analogues. The chemical structures of these nonbasic metabolites have been elucidated by spectroscopic means and supported by independent syntheses. Their chemical properties as well as their effects on plant and human physiology have been under intense scrutiny in the last few decades, and significant knowledge has been gained. Yet, none of these compounds has advanced to human clinical trials for treating cancer, and there is still a lot to be done before this happens. We finish our review by identifying further possible research directions that will bring us closer to the imple-

Scheme 20^a

^a Reagents and conditions: (a) AcOH, 100 °C. (b) MgBr₂·OEt₂, COCl₂, ether, **219**. (c) K₂CO₃, MOMCl, DMF. (d) *t*-BuLi, CeCl₃, ultrasound, THF, -78 °C to room temperature. (e) BBr₃, CH₂Cl₂, -78 °C, then MeOH, -78 °C to room temperature.

mentation of these compounds and/or their derivatives in human medicine.

The apparent major impediment thwarting further developments in the preclinical evaluation of these compounds is the issue of supply. Because isolated amounts of these isoquinolines from the discovered plant sources are small, further search for plants synthesizing these metabolites is essential. In this context, the identification of narciclasine and pancratistatin in the lubber Grasshopper *B. Magna*, collected in Texas, is intriguing.⁷⁵ It is believed that insects select their diet to obtain plant constituents important for their own chemical defense.²²⁶ Surprisingly, species of the Amaryllidaceae family have not been recorded among this Grasshopper's preferred foods.²²⁷ This finding may have potentially significant implications in the discovery of new plant sources for these anticancer isocarbostryls.

Additionally, a practical chemical synthesis from a commercially available inexpensive starting material constitutes a very important alternative. It is hoped that researchers, who report such multistep synthetic pathways, revisit their work again and again developing second and third generation syntheses in pursuit of practicality and scalability. Synthesis and testing of structurally simplified analogues of natural compounds, which may be more amenable to large-scale preparation, is another important research direction. Rather surprisingly, so far, such efforts have not resulted in compounds with simpler structures but comparable medicinal potential. This work, however, is quite empirical in character due to our poor understanding of the biological mechanism(s) of action associated with the Amaryllidaceae anticancer isocarbostryls. The mechanistic studies, revealing inhibition of protein biosynthesis by narciclasine and some of its natural and synthetic congeners, as discussed in this review, are 30 years old. There has been very little new research done in this area since then. Undoubtedly, the insights that may be gained from the structural studies of these isocarbostryls

and their ribosomal binding site(s) will have a tremendous impact on our understanding of the bioactive pharmacophore(s) associated with these compounds. Such knowledge will guide more successful analogue generation efforts.

Lastly, natural products are often referred to as evolutionarily selected privileged medicinal structures.^{228–230} In the process of their biosynthesis, they interact with many biological macromolecules; thus, they are likely to bind to a variety of structurally and functionally discreet receptors. It follows that natural products may manifest multiple biological activities or exhibit the same activity through a number of different mechanisms. Examples of such observations abound in the literature. In this respect, preliminary accounts of the preclinical study of pancratistatin phosphate prodrugs reported by Pettit and co-workers are intriguing and indicate that the mechanism of action of pancratistatin against *in vivo* antineoplastic disease involves at least in part angiogenesis/antivascular targeting.^{179,180} Whether this effect is related to the inhibition of protein biosynthesis in the vascular endothelial cells or is due to an entirely different mode of action is one of the many stimulating questions that will draw interest and incite further research efforts in this area. We are looking forward to updating this review in a few years time, hopefully, with the results of the clinical trials of the Amaryllidaceae anticancer constituents or their synthetic analogues.

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12. References

- Hartwell, J. L. *Lloydia* **1967**, *30*, 379.
- Gardeil, J. B., translator. *Traduction des Oeuvres Médicales d'Hippocrate*, Fages, Meilhec et Cie: Toulouse, 1801; 4 volumes.
- Aetios of Amida. *The Gynaecology and Obsterics of the VI Century, A.D.*; Blakiston Co.: Philadelphia, 1950; 215 pp, translated by J. V. Ricci.
- Ibn al-Baitar. Leclerc, L. *Traité des Simples par Ibn el-Beithar, Notices et Extraits des Manuscrits de la Bibl. Nat.*: Paris, 1877; 23 (I), 476 pp; 1881, 25 (I), 489 pp; 1883, 26 (I), 483 pp.
- Martin, S. F. *The Alkaloids*; Academic Press: New York, 1987; p 251.
- Gibbs, R. D. *Chemotaxonomy of Flowering Plants*; McGill-Queen's University Press: Montreal, 1974; Vol. III, p 1924.
- Tojo, E. *J. Nat. Prod.* **1991**, *54*, 1387.
- Noumbissie, B. E.; Kapnang, H.; Fomum, Z. T.; Martin, M.-T.; Bodo, B. *J. Nat. Prod.* **1992**, *55*, 137.
- Bastida, J.; Codina, C.; Viladomat, F.; Rubiralta, M.; Quirion, J.-C.; Weniger, B. *J. Nat. Prod.* **1992**, *55*, 122.
- Plinius Secundus C. (Pliny the Elder). *Natural History*; translated by Bostock, J., and Riley, H. T.; Bohn: London, 1855; 6 volumes.
- Duke, J. A.; Duke, P. K. *Medicinal Plants of the Bible*; Trad-Medic Books: New York, 1983; p 98.
- Hartwell, J. L. *Plants Used Against Cancer*; Quarterman Publication: Lawrence, MA, 1982.
- Kosteletzky, B. F. *Allgemeine Medizinisch-Pharmazeutische Flora*; Borrosch and André: Prague, 1831–1836; 6 volumes.
- Cook, J. W.; Loudon, J. D. *The Alkaloids*; Academic Press: New York, 1952; p 331.
- Piozzi, F.; Marino, M. L.; Fuganti, C.; Di Martino, A. *Phytochemistry* **1969**, *8*, 1745.
- Rinner, U.; Hudlicky, T. *Synlett* **2005**, 365.
- Chapleur, Y.; Chrétien, F.; Ibn-Ahmed, S.; Khaldi, M. *Curr. Org. Synth.* **2006**, *3*, 341.
- Louw, C. A. M.; Regnier, T. J. C.; Korsten, L. *J. Ethnopharmacol.* **2002**, *82*, 147.

- (19) Nakagawa, Y.; Uyeo, S.; Yayima, H. *Chem. Ind.* **1956**, 1238.
- (20) Arrigoni, O.; Arrigoni Liso, R.; Calabrese, G. *Nature* **1975**, *256*, 513.
- (21) Evidente, A.; Motta, A. *2001 Studies in Natural Products Chemistry*; Elsevier: Amsterdam, 2002, p 581.
- (22) Ghosal, S.; Saini, K. S.; Razdan, S. *Phytochemistry* **1985**, *24*, 2141.
- (23) Wildman, W. C. *The Alkaloids*; Academic Press: New York, 1960; p 289.
- (24) Wildman, W. C. *The Alkaloids*; Academic Press: New York, 1968; p 307.
- (25) Fuganti, C. *The Alkaloids*; Academic Press: New York, 1975; p 83.
- (26) Suffness, M.; Cordell, G. A. *The Alkaloids*; Academic Press: New York, 1985; p 3.
- (27) Bastida, J.; Viladomat, F.; Codina, C. *Studies in Natural Products Chemistry*; Elsevier: Amsterdam, 1998; p 323.
- (28) Evidente, A. *Flavour and Fragrance*; Kluwer: Dordrecht, 2000; p 109.
- (29) Bezhenova, E. D.; Aliev, K. V.; Zairov, V. B. *Farmakol. Alkaloid Ser. Glik.* **1972**, 100.
- (30) Zakirov, U. B.; Umarova, S. S. *Farmakol. Alkaloid Ser. Glik.* **1971**, 96.
- (31) Ieven, M.; VandenBerghe, D. A.; Mertens, I.; Vlietinck, A. J.; Lammens, E. *Planta Med.* **1979**, *36*, 311.
- (32) Pettit, G. R.; Gaddamidi, V.; Goswami, A.; Cragg, G. M. *J. Nat. Prod.* **1984**, *47*, 796.
- (33) Evidente, A.; Andolfi, A.; Abou-Donia, A. H.; Touema, S. M.; Hammoda, H. M.; Shawky, E.; Motta, A. *Phytochemistry* **2004**, *65*, 2113.
- (34) Ali, A. A.; Ross, S. A.; El-Moghazy, A.; El-Moghazy, S. A. *J. Nat. Prod.* **1983**, *46*, 350.
- (35) Jeffs, P. W.; Mueller, L.; Abou-Donia, A. H.; Seif El-Din, A. A.; Campau, D. *J. Nat. Prod.* **1988**, *51*, 549.
- (36) Evidente, A.; Andolfi, A.; Abou-Donia, A. H.; Darwish, F. A.; Hammoda, H. A. M.; Motta, A. *Alexandria J. Pharm. Sci.* **2005**, *19*, 49.
- (37) Evidente, A.; Abou-Donia, A. H.; Darwish, F. A.; Amer, M. E.; Kassem, F. F.; Hammoda, H. A. M.; Motta, A. *Phytochemistry* **1999**, *51*, 1151.
- (38) Fennell, C. W.; van Staden, J. *J. Ethnopharmacol.* **2001**, *78*, 15.
- (39) Ghosal, S.; Saini, K. S.; Frahm, A. W. *Phytochemistry* **1983**, *22*, 2305.
- (40) Houghton, P. J.; Agbedahunsi, J. M.; Adegbulugbe, A. *Phytochemistry* **2004**, *65*, 2893.
- (41) Elgorashi, E. E.; van Staden, J. *J. Ethnopharmacol.* **2004**, *90*, 27.
- (42) Ramanathan, S.; Furusawa, E.; Kroposki, M.; Furusawa, S.; Cutting, W. *Chemotherapy* **1968**, *13*, 121.
- (43) Abou-Donia, A. H.; Darwish, F. A.; Ghazy, N. M. *Alexandria J. Pharm. Sci.* **1989**, *3*, 122.
- (44) Evidente, A.; Lanzetta, R.; Abou-Donia, A. H.; Amer, M. E.; Kassem, F. F.; Harraz, F. M. *Arch. Pharm.* **1994**, *327*, 595.
- (45) Sener, B.; Orhan, I.; Satayavivad, J. *Phytother. Res.* **2003**, *17*, 1220.
- (46) Ghosal, S.; Saini, K. S.; Rao, P. H.; Kumar, Y.; Jaiswal, D. K.; Chattopadhyay, S. *Proc. 6th IndoSoviet Symp., Chemistry of Natural Products*; NCL: Pune, India, 1981; p 71.
- (47) Ali, A. A.; Mesbah, M. K.; Mohamed, M. H. *Bull. Pharm. Sci. Assiut Univ.* **1984**, *7*, 351.
- (48) Sandberg, F.; Michel, K. H. *Acta Pharm. Suecica* **1968**, *5*, 61.
- (49) Vasquez Tato, M. P.; Castedo, L.; Riguera, R. *Heterocycles* **1988**, *27*, 2833.
- (50) Abou-Donia, A. H.; Abib, A.-A.; Seif El Din, A.; Evidente, A.; Gaber, M.; Scopa, A. *Hytochemistry* **1992**, *31*, 2139.
- (51) Ghosal, S.; Singh, S. K.; Srivastava, R. S. *Phytochemistry* **1986**, *25*, 1975.
- (52) Ghosal, S.; Singh, S. K.; Kumar, Y.; Unnikrishnan, S.; Chattopadhyay, S. *Planta Med.* **1988**, *54*, 114.
- (53) Evidente, A.; Randazzo, G.; Surico, G.; Lavermicocca, P.; Arrigoni, O. *J. Nat. Prod.* **1985**, *48*, 564.
- (54) Evidente, A.; Iasiello, I.; Randazzo, G. *J. Nat. Prod.* **1984**, *47*, 1003.
- (55) Normatov, M.; Abduazimov, Kh. A.; Yunusov, S. Yu. *Uzbeksk. Khim. Zh.* **1965**, *9*, 25.
- (56) Ali, A. A.; El-Sayed, H. M.; Abdallah, O. M.; Steglich, W. *Phytochemistry* **1986**, *25*, 2399.
- (57) Ghosal, S.; Kumar, Y.; Singh, S. K.; Kumar, A. *J. Chem. Res., Synop.* **1986**, 112.
- (58) Watt, J. W.; Breyer-Brandwijk, M. G. *Medicinal and Poisonous Plants of Southern and Eastern Africa*; Livingstone, E. and S. Ltd.: London, 1962; p42.
- (59) Ghosal, S.; Singh, S. K.; Srivastava, R. S. *Phytochemistry* **1985**, *24*, 151.
- (60) Ghosal, S.; Ashutosh; Radzan, S. *Phytochemistry* **1985**, *24*, 635.
- (61) Scott, G. *Veld Flora* **1993**, *79*, 84.
- (62) Hutchings, A.; Scott, A. H.; Lewis, G.; Cunningham, A. B. *Zulu Medicinal Plants: An Inventory*; University Natal Press: Pietermaritzburg, 1996.
- (63) Piozzi, F.; Fuganti, C.; Mondelli, R.; Cerotti, G. *Tetrahedron* **1968**, *24*, 1119.
- (64) Ceriotti, G. *Nature* **1967**, *213*, 595.
- (65) Savona, G.; Piozzi, F.; Marino, M. L. *J. Chem. Soc., Chem. Commun.* **1970**, 1006.
- (66) Immirzi, A.; Fuganti, C. *J. Chem. Soc., Chem. Commun.* **1972**, 240.
- (67) Okamoto, T.; Torii, Y.; Isogai, Y. *Chem. Pharm. Bull.* **1968**, *16*, 1860.
- (68) Iraida, S.; Zaida, T. *Revista Cubana de Farmacia* **1989**, *23*, 151; journal written in Spanish; CAN 112:115819 AN 1990:115819.
- (69) Shuzo, T.; Masae, Y. *Yakugaku Zasshi* **1974**, *94*, 617.
- (70) Pettit, G. R.; Gaddamidi, V.; Herald, D. L.; Singh, S. B.; Gragg, G. M.; Schimdt, J. M.; Boettner, F. E.; William, M.; Sagawa, Y. *J. Nat. Prod.* **1986**, *49*, 995.
- (71) Ghosal, S.; Singh, S.; Kumar, Y.; Srivastava, R. S. *Phytochemistry* **1989**, *28*, 611.
- (72) Pettit, G. R.; Pettit, G. R., III; Backhaus, R. A.; Boyd, M. R.; Meerow, A. W. *J. Nat. Prod.* **1993**, *56*, 1682.
- (73) Pettit, G. R.; Gaddamidi, V.; Cragg, G. M.; Herald, D. L.; Sagawa, Y. *J. Chem. Soc., Chem. Commun.* **1984**, 1693.
- (74) Pettit, G. R.; Cragg, G. M.; Singh, S. B.; Duke, J. A.; Doubek, D. L. *J. Nat. Prod.* **1990**, *53*, 176.
- (75) Pettit, G. R.; Meng, Y.; Herald, D. L.; Knight, J. C.; Day, J. F. *J. Nat. Prod.* **2005**, *68*, 1256.
- (76) Ghosal, S.; Lochan, R.; Ashutosh; Kumar, Y.; Srivastava, R. S. *Phytochemistry* **1985**, *24*, 1825.
- (77) Abou-Donia, A. H.; De Giulio, A.; Evidente, A.; Gaber, M.; Habib, A.-A.; Lanzetta, R.; Seif El Din, A. A. *Phytochemistry* **1991**, *30*, 3445.
- (78) Ghosal, S.; Datta, K.; Singh, S. K.; Kumar, Y. *J. Chem. Res.* **1990**, 334.
- (79) Kojima, K.; Mutsuga, M.; Inoue, M.; Ogihara, Y. *Phytochemistry* **1998**, *48*, 1199.
- (80) Pettit, G. R.; Gaddamidi, V.; Gragg, G. M. *J. Nat. Prod.* **1984**, *47*, 1018.
- (81) Bi, Y. R.; Yung, K.-H.; Wong, Y.-S. *Plant Sci.* **1998**, *135*, 103.
- (82) Pettit, G. R.; Pettit, G. R., III; Groszek, G.; Backhaus, R. A.; Doubek, D. L.; Barr, R. J. *J. Nat. Prod.* **1995**, *58*, 756.
- (83) Pettit, G. R.; Pettit, G. R., III; Backhaus, R. A.; Boettner, F. E. *J. Nat. Prod.* **1995**, *58*, 37.
- (84) Pettit, G. R.; Eastham, S. A.; Melody, N.; Orr, B.; Herald, D. L.; McGregor, J.; Knight, J. C.; Doubek, D. L.; Pettit, G. R., III; Garner, L. C.; Bell, J. A. *J. Nat. Prod.* **2006**, *69*, 7.
- (85) Evidente, A. *Planta Med.* **1991**, *57*, 293.
- (86) Bernfeld, P. *Biogenesis of Natural Compounds*; Pergamon Press: Oxford, 1963; p 985.
- (87) Battersby, A. R.; Herbert, R. B.; McDonald, E.; Ramage, R.; Clements, J. H. *Chem. Commun.* **1966**, 603.
- (88) Battersby, A. R.; Binks, R.; Breuer, S. W.; Fales, H. M.; Wildman, W. C.; Hight, R. J. *J. Chem. Soc.* **1964**, 1595.
- (89) Battersby, A. R.; Binks, R.; Breuer, S. W.; Fales, H. M.; Wildman, W. C. *Proc. Chem. Soc.* **1961**, 243.
- (90) Kirby, G. W.; Tiwari, H. P. *J. Chem. Soc. (C)* **1966**, 676.
- (91) Barton, D. H. R.; Kirby, G. W.; Taylor, J. B.; Thomas, G. M. *Proc. Chem. Soc.* **1962**, 179.
- (92) Barton, D. H. R.; Kirby, G. W.; Taylor, J. B.; Thomas, G. M. *J. Chem. Soc.* **1963**, 4545.
- (93) Battersby, A. R.; Fales, H. M.; Wildman, W. C. *J. Am. Chem. Soc.* **1961**, *83*, 4098.
- (94) Jeffs, P. W. *Proc. Chem. Soc.* **1962**, 80.
- (95) Fuganti, C. *Tetrahedron Lett.* **1973**, *14*, 1785.
- (96) Murphy, C. F.; Wildman, W. C. *Tetrahedron Lett.* **1964**, *5*, 3863.
- (97) Barton, D. H. R. *Proc. Chem. Soc.* **1963**, 293.
- (98) Barton, D. H. R. *Pure Appl. Chem.* **1964**, *9*, 35.
- (99) Barton, D. H. R.; Kirby, G. W. *Proc. Chem. Soc.* **1960**, 392.
- (100) Fuganti, C.; Staunton, J.; Battersby, A. R. *Chem. Commun.* **1971**, 1154.
- (101) Fuganti, C.; Mazza, M. *Chem. Commun.* **1971**, 1388.
- (102) Battersby, A. R.; Hebert, R. B.; McDonald, E.; Ramage, R.; Clements, J. H. *Chem. Commun.* **1966**, 603.
- (103) Fuganti, C. *Gazz. Chim. Ital.* **1973**, *103*, 1255.
- (104) Backhaus, R. A.; Pettit, G. R., III; Huang, D. S.; Pettit, G. R.; Groszek, G.; Odgers, J. C.; Ho, J.; Meerow, A. *Acta Hort.* **1992**, 364-366.
- (105) Mondon, A.; Krohn, K. *Chem. Ber.* **1975**, *108*, 445.
- (106) Pettit, G. R.; Melody, N.; O'Sullivan, M.; Thompson, M. A.; Herald, D. L.; Coates, B. *J. Chem. Soc., Chem. Commun.* **1994**, 2725.
- (107) Pettit, G. R.; Melody, N.; Herald, D. L.; Schmidt, J. M.; Pettit, R. K.; Chapuis, J. C. *Heterocycles* **2002**, *56*, 139.
- (108) Pettit, G. R.; Melody, N.; Herald, D. L.; Knight, J. C.; Chapuis, J. C. *J. Nat. Prod.* **2007**, *70*, 417.
- (109) Pettit, G. R.; Melody, N.; Herald, D. L. *J. Org. Chem.* **2001**, *66*, 2583.
- (110) Carrasco, L.; Fresno, M.; Vazquez, D. *FEBS Lett.* **1975**, *52*, 236.

- (111) Jimenez, A.; Sanchez, L.; Vazquez, D. *FEBS Lett.* **1975**, *55*, 53.
- (112) Jimenez, A.; Sanchez, L.; Vazquez, D. *FEBS Lett.* **1975**, *60*, 66.
- (113) Jimenez, A.; Santos, A.; Alonso, G.; Vazquez, D. *Biochim. Biophys. Acta* **1976**, *425*, 342.
- (114) Baez, A.; Angoso, M.; Alonso, G.; Vazquez, D. *Biochimie* **1977**, *59*, 751.
- (115) Baez, A.; Vazquez, D. *Biochim. Biophys. Acta* **1978**, *518*, 95.
- (116) Barendse, L. V. J. *Vakblad voor de Bloemisterij* **1974**, *29*, 12.
- (117) van Doorn, W. G. *J. Am. Hortic. Sci.* **1998**, *123*, 146.
- (118) van Doorn, W. G.; Sinz, A.; Tomassen, M. M. *Phytochemistry* **2004**, *65*, 571.
- (119) Sasse, J. M.; Wardrop, J. J.; Rowan, K. S.; Aspinall, D.; Coombe, B. G.; Paleg, L. G.; Buta, J. G. *Physiol. Plant.* **1982**, *55*, 51.
- (120) Sasse, J. M.; Galbraith, M. N.; Horn, D. H. S.; Adamson, D. A. *Plant Growth Substances*; Springer-Verlag: Berlin, 1972; pp 299–305.
- (121) Katekar, G. F.; Geissler, A. E. *Plant Physiol.* **1980**, *66*, 1190.
- (122) Isogai, Y.; Nomoto, S.; Noma, T.; Okamoto, T. *Plant Growth Substances*; Hirokawa Publishing Co.: Tokyo, 1974; pp 9–14.
- (123) Edelbluth, E.; Kaldewey, H. *Planta* **1976**, *131*, 285.
- (124) Kang, B. G.; Lee, J. S.; Oh, S. E.; Horiuchi, Y.; Imaseki, H. *Plant Cell Physiol.* **1984**, *25*, 249.
- (125) Sasse, J. M.; Cerana, R.; Colombo, R. *Physiol. Plant.* **1984**, *62*, 303.
- (126) Kaldewey, H.; Ginkel, U. *Plant Growth Substances*; Hirokawa Publishing Co.: Tokyo, 1974; pp 1079–1089.
- (127) Bi, Y. R.; Zhang, L. X.; Guo, J. K.; Yung, K. H.; Wong, Y. S. *N. Z. J. Crop. Hortic. Sci.* **2003**, *31*, 335.
- (128) Numata, A.; Takemura, T.; Ohbayashi, H.; Katsuno, T.; Yamamoto, K.; Sato, K.; Kobayashi, S. *Chem. Pharm. Bull.* **1983**, *31*, 2146.
- (129) Evidente, A.; Arrigoni, O.; Liso, R.; Calabrese, G.; Randazzo, G. *Phytochemistry* **1986**, *25*, 2739.
- (130) Ghosal, S.; Shanthi, A.; Kumar, A.; Kumar, Y. *Phytochemistry* **1985**, *24*, 2703.
- (131) Ghosal, S.; Kumar, Y.; Singh, S. *Phytochemistry* **1984**, *23*, 1167.
- (132) Green, R. I.; Greenberg, N. H.; MacDonald, M. M.; Schumacher, A. M.; Abbott, B. J. *Cancer Chemother. Rep.* **1972**, *3*, 1.
- (133) Nakayama, M.; Horie, T.; Tsukayama, M.; Masumura, M.; Hayashi, S. *Z. Naturforsch., C: J. Biosci.* **1978**, *33*, 587.
- (134) Pettit, G. R.; Fujii, Y.; Hasler, J. A.; Schmidt, J. M.; Michel, C. J. *Nat. Prod.* **1982**, *45*, 263.
- (135) Paull, K. D.; Shoemaker, R. H.; Hodes, L.; Monks, A.; Scudiero, D. A.; Rubinstein, L.; Plowman, J.; Boyd, M. R. *J. Natl. Cancer Inst.* **1989**, *81*, 1088.
- (136) Mutsuga, M.; Kojima, K.; Yamashita, M.; Ohno, T.; Oghihara, Y.; Inoue, M. *Biol. Pharm. Bull.* **2002**, *25*, 223.
- (137) Hudlicky, T.; Moser, M.; Banfield, S. C.; Rinner, U.; Chapuis, J.-C.; Pettit, G. R. *Can. J. Chem.* **2006**, *84*, 1313.
- (138) Kekre, N.; Griffin, C.; McNulty, J.; Pandey, S. *Cancer Chemother. Pharmacol.* **2005**, *56*, 29.
- (139) Pandey, S.; Kekre, N.; Naderi, J.; McNulty, J. *Artif. Cells, Blood Substitutes, Immobilization Biotechnol.* **2005**, *33*, 279.
- (140) McLachlan, A.; Kekre, N.; McNulty, J.; Pandey, S. *Apoptosis* **2005**, *10*, 619.
- (141) Rinner, U.; Hillebrenner, H. L.; Adams, D. R.; Hudlicky, T.; Pettit, G. R. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 2911.
- (142) Blaskó, G.; Major, E.; Blaskó, G.; Rószka, I.; Szántay, C. *Eur. J. Med. Chem.* **1986**, *21*, 91.
- (143) Rinner, U.; Hudlicky, T.; Gordon, H.; Pettit, G. R. *Angew. Chem., Int. Ed.* **2004**, *43*, 5342.
- (144) Hudlicky, T.; Rinner, U.; Finn, K. J.; Ghiviriga, I. *J. Org. Chem.* **2004**, *70*, 3490.
- (145) Moser, M.; Sun, X.; Hudlicky, T. *Org. Lett.* **2005**, *7*, 5669.
- (146) Hudlicky, T.; Rinner, U.; Gonzales, D.; Akgun, H.; Schilling, S.; Siengalewicz, P.; Martinot, T. A.; Pettit, G. R. *J. Org. Chem.* **2002**, *67*, 8726.
- (147) Ibn-Ahmed, S.; Khaldi, M.; Chrétien, F.; Chapleur, Y. *J. Org. Chem.* **2004**, *69*, 6722.
- (148) Chrétien, F.; Ibn-Ahmed, S.; Masion, A.; Chapleur, Y. *Tetrahedron* **1993**, *49*, 7463.
- (149) Phung, A. N.; Zannetti, M. T.; Whited, G.; Fessner, W.-D. *Angew. Chem. Int. Ed.* **2003**, *42*, 4821.
- (150) Nadein, O. N.; Kornienko, A. *Org. Lett.* **2004**, *6*, 831.
- (151) Kireev, A. S.; Nadein, O. N.; Agustin, V. J.; Bush, N. E.; Evidente, A.; Manpadi, M.; Ogasawara, M. A.; Rastogi, S. K.; Rogel, S.; Shors, S. T.; Kornienko, A. *J. Org. Chem.* **2006**, *71*, 5694.
- (152) Lopes, R. S.; Lopes, C. C.; Heathcock, C. H. *Tetrahedron Lett.* **1992**, *33*, 6775.
- (153) Rigby, J. H.; Gupta, V. *Synlett* **1995**, 547.
- (154) Banwell, M. G.; Cowden, C. J.; Mackay, M. F. *J. Chem. Soc., Chem. Commun.* **1994**, 61.
- (155) Banwell, M. G.; Cowden, C. J.; Gable, R. W. *J. Chem. Soc., Perkin Trans. 1* **1994**, 3515.
- (156) McNulty, J.; Mao, J.; Gibe, R.; Mo, R.; Wolf, S.; Pettit, G. R.; Herald, D. L.; Boyd, M. R. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 169.
- (157) McNulty, J.; Larichev, V.; Pandey, S. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 5315.
- (158) Pandey, G.; Murugan, A.; Balakrishnan, M. *J. Chem. Soc., Chem. Commun.* **2002**, 624.
- (159) Shukla, K. H.; Boehmler, D. J.; Bogaczyk, S.; Duvall, B. R.; Peterson, W. A.; McElroy, W. T.; DeShong, P. *Org. Lett.* **2006**, *8*, 4183.
- (160) Ortiz, J. C.; Ozores, L.; Cagide-Fagin, F.; Alonso, R. *J. Chem. Soc., Chem. Commun.* **2006**, 4239.
- (161) Keck, G. E.; Wager, T. T. *J. Org. Chem.* **1996**, *61*, 8366.
- (162) Keck, G. E.; Wager, T. T.; Rodriguez, J. F. D. *J. Am. Chem. Soc.* **1999**, *121*, 5176.
- (163) Akgün, H.; Hudlicky, T. *Tetrahedron Lett.* **1999**, *40*, 3081.
- (164) Gabrielsen, B.; Monath, T. P.; Huggins, J. W.; Kefauver, D. F.; Pettit, G. R.; Groszek, G.; Hollingshead, M.; Kirsj, J. J.; Shannon, W. M.; Shubert, E. M.; Dare, J.; Ugarkar, B.; Ussery, M. A.; Phelan, M. J. *J. Nat. Prod.* **1992**, *55*, 1569.
- (165) Gabrielsen, B.; Monath, T. P.; Huggins, J. W.; Kirsj, J. J.; Hollingshead, M.; Shannon, W. M.; Pettit, G. R. *Natural Products as Antiviral Agents*; Plenum Press: New York, 1992; p 121.
- (166) Sidwell, R. W.; Huffman, J. H.; Barnard, D. L.; Smee, D. F.; Warren, R. P.; Chirigos, M. A.; Kende, M.; Huggins, J. *Antiviral Res.* **1994**, *25*, 105.
- (167) Furusawa, E.; Irie, H.; Combs, D.; Wildman, W. C. *Chemotherapy* **1980**, *26*, 36.
- (168) Quarzane-Amara, M.; Franetich, J.-F.; Mazier, D.; Pettit, G. R.; Meijer, L.; Doerig, C.; Desportes-Livage, I. *Antimicrob. Agents Chemother.* **2001**, *45*, 3409.
- (169) Fox, B. W. *Trans. R. Soc. Trop. Med. Hyg.* **1991**, *85*, 22.
- (170) Veronese, F. M.; Ceriotti, G.; Keller, G.; Lora, S.; Carenza, M. *Radiat. Phys. Chem.* **1990**, *35*, 88.
- (171) Veronese, F. M.; Ceriotti, G.; Caliceti, P.; Lora, S.; Carenza, M. *J. Controlled Release* **1991**, *16*, 291.
- (172) Beijnen, J. H.; Flora, K. P.; Halbert, G. W.; Henrar, R. E. C.; Slack, J. A. *Br. J. Cancer* **1995**, *72*, 210.
- (173) Torres-Labandeira, J. J.; Davignon, P.; Pitha, J. *J. Pharm. Sci.* **1991**, *80*, 384.
- (174) Pettit, G. R.; Freeman, S.; Simpson, M. J.; Thompson, M. A.; Boyd, M. R.; Williams, M. D.; Pettit, G. R., III; Doubek, D. L. *Anti-Cancer Drug Des.* **1995**, *10*, 243.
- (175) Pettit, G. R.; Orr, B.; Ducki, S. *Anti-Cancer Drug Des.* **2000**, *15*, 389.
- (176) Pettit, G. R.; Melody, N.; Simpson, M.; Thompson, M.; Herald, D. L.; Knight, J. C. *J. Nat. Prod.* **2003**, *66*, 92.
- (177) Pettit, G. R.; Melody, N.; Herald, D. L. *J. Nat. Prod.* **2004**, *67*, 322.
- (178) Pettit, G. R.; Melody, N. *J. Nat. Prod.* **2005**, *68*, 207.
- (179) Bibby, M. C.; Holwell, S. E.; Pettit, G. R. *Anti-Vascular and Anti-Tumor Effects of the Novel Agent Pancratistatin Phosphate*; Biological Basis for Antiangiogenic Therapy Conference, Milan, Italy, November 8–10, 1999.
- (180) Shnyder, S. D.; Cooper, P. A.; Millington, N. J.; Gill, J. H.; Pettit, G. R.; Bibby, M. C. *Clin. Cancer Res.* **2005**, *11* (24 Suppl.), 8971s.
- (181) Polt, R. *Amaryllidaceae alkaloids with antitumor activity*. In *Organic Synthesis: Theory and Applications*; Jai Press Inc.: Greenwich, CT, 1997; Vol. 3, pp 109–148.
- (182) Hudlicky, T. *Chem. Rev.* **1996**, *96*, 3.
- (183) Rigby, J. H.; Mateo, M. E. *J. Am. Chem. Soc.* **1997**, *119*, 12655.
- (184) Rigby, J. H.; Maharoo, U. S. M.; Mateo, M. E. *J. Am. Chem. Soc.* **2000**, *122*, 6624.
- (185) Gonzalez, D.; Martinot, T.; Hudlicky, T. *Tetrahedron Lett.* **1999**, *40*, 3077.
- (186) Elango, S.; Yan, T.-H. *J. Org. Chem.* **2002**, *67*, 6954.
- (187) Danishefsky, S.; Lee, J. Y. *J. Am. Chem. Soc.* **1989**, *111*, 4829.
- (188) Tian, X.; Hudlicky, T.; Königsberger, K. *J. Am. Chem. Soc.* **1995**, *117*, 3643.
- (189) Hudlicky, T.; Tian, X.; Königsberger, K.; Maurya, R.; Rouden, J.; Fan, B. *J. Am. Chem. Soc.* **1996**, *118*, 10752.
- (190) Trost, B. M.; Pulley, S. R. *J. Am. Chem. Soc.* **1995**, *117*, 10143.
- (191) Doyle, T. J.; Hendrix, M.; VanDerveer, D.; Javanmard, S.; Haseltine, J. *Tetrahedron* **1997**, *53*, 11153.
- (192) Magnus, P.; Sebbat, I. K. *J. Am. Chem. Soc.* **1998**, *120*, 5341.
- (193) Magnus, P.; Sebbat, I. K. *Tetrahedron* **1998**, *54*, 15509.
- (194) Kim, S.; Ko, H.; Kim, E.; Kim, D. *Org. Lett.* **2002**, *44*, 1343.
- (195) Ko, H.; Kim, E.; Park, J. E.; Kim, D.; Kim, S. *J. Org. Chem.* **2004**, *69*, 112.
- (196) Li, M.; Wu, A.; Zhou, P. *Tetrahedron Lett.* **2006**, *47*, 3707.
- (197) Ohta, S.; Kimoto, S. *Tetrahedron Lett.* **1975**, 2279.
- (198) Ohta, S.; Kimoto, S. *Chem. Pharm. Bull.* **1976**, *24*, 2969.
- (199) Ohta, S.; Kimoto, S. *Chem. Pharm. Bull.* **1976**, *24*, 2977.
- (200) Paulsen, H.; Stubbe, M. *Tetrahedron Lett.* **1982**, *23*, 3171.
- (201) Paulsen, H.; Stubbe, M. *Liebigs Ann. Chem.* **1983**, 535.
- (202) Ugarkar, B. G.; DaRe, J.; Schubert, E. M. *Synthesis* **1987**, 715.
- (203) Chida, N.; Ohtsuka, M.; Ogawa, S. *Tetrahedron Lett.* **1991**, *32*, 4525.
- (204) Chida, N.; Ohtsuka, M.; Ogawa, S. *J. Org. Chem.* **1993**, *58*, 4441.

- (205) Hudlicky, T.; Olivo, H. F. *J. Am. Chem. Soc.* **1992**, *114*, 9694.
- (206) Hudlicky, T.; Olivo, H. F.; McKibben, B. *J. Am. Chem. Soc.* **1994**, *116*, 5108.
- (207) Martin, S. F.; Tso, H.-H. *Heterocycles* **1993**, *35*, 85.
- (208) Elango, S.; Yan, T.-H. *Tetrahedron* **2002**, *58*, 7335.
- (209) Zhang, H.; Padwa, A. *Org. Lett.* **2006**, *8*, 247.
- (210) Padwa, A.; Zhang, H. *J. Org. Chem.* **2007**, *72*, 2570.
- (211) Keck, G. E.; McHardy, S. F.; Murry, J. A. *J. Am. Chem. Soc.* **1995**, *117*, 7289.
- (212) Keck, G. E.; McHardy, S. F.; Murry, J. A. *J. Org. Chem.* **1999**, *64*, 4465.
- (213) Tian, X.; Maurya, R.; Königsberger, K.; Hudlicky, T. *Synlett* **1995**, 1125.
- (214) Chida, N.; Jitsuoka, M.; Yamamoto, Y.; Ohtsuka, M.; Ogawa, S. *Heterocycles* **1996**, *43*, 1385.
- (215) Keck, G. E.; Wager, T. T.; McHardy, S. F. *J. Org. Chem.* **1998**, *63*, 9164.
- (216) Aceña, J. L.; Arjona, O.; León, M. L.; Plumet, J. *Org. Lett.* **2000**, *2*, 3683.
- (217) Håkansson, A. E.; Palmelund, A.; Holm, H.; Madsen, R. *Chem. Eur. J.* **2006**, *12*, 3243.
- (218) Zhang, H.; Padwa, A. *Tetrahedron Lett.* **2006**, *47*, 3905.
- (219) Shin, I.-J.; Choi, E.-S.; Cho, C.-G. *Angew. Chem., Int. Ed.* **2007**, *46*, 2303.
- (220) Isobe, K.; Taga, J.-I.; Tsuda, Y. *Heterocycles* **1978**, *9*, 625.
- (221) Fujimura, T.; Shibuya, M.; Ogasawara, K.; Iwabuchi, Y. *Heterocycles* **2005**, *66*, 167.
- (222) Hudlicky, T.; Boros, E. E.; Boros, C. H. *Synthesis* **1992**, 174.
- (223) Ikeda, M.; El-Bialy, S. A. A.; Yakura, T. *Heterocycles* **1999**, *51*, 1957.
- (224) Lautens, M.; Fang, Y.-Q. *Org. Lett.* **2003**, *5*, 3679.
- (225) Rinner, U.; Siengalewicz, P.; Hudlicky, T. *Org. Lett.* **2002**, *4*, 115.
- (226) Bright, K. L.; Bernays, E. A.; Moran, V. C. *J. Insect Behav.* **1994**, *7*, 779.
- (227) Bernays, E. A.; Bright, K. L. *Comp. Biochem. Physiol.* **1993**, *104A*, 125.
- (228) Tan, D. S. *Comb. Chem. High Throughput Screening* **2004**, *7*, 631.
- (229) Boldi, A. M. *Curr. Opin. Chem. Biol.* **2004**, *8*, 281.
- (230) Liao, Y.; Hu, Y.; Wu, J.; Zhu, Q.; Donovan, M.; Fathi, R.; Yang, Z. *Curr. Med. Chem.* **2003**, *10*, 2285.

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