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 (a.k.a. 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 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 thirty other plants of the Amaryllidaceae family, in folk medicine for the management of cancer[1] speaks volumes to validate this conjecture.

Indeed, powerful anticancer properties of *Narcissus poeticus* L. were already known to the Father of Medicine, Hippokrates 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 *N. 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 *N. 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, e. g. *Hymenocallis caribaea* (L. emend Gawler) Herbert, utilized by early European medical practitioners for inflammatory tumors.[13]

More recently, the plants of the 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 *Narcissus pseudonarcissus*[14] and since then more than 100 alkaloids, exhibiting diverse biological activities, have been isolated from the Amaryllidaceae plants.

Based on the present scientific evidence, it is likely that isocarbostyril constituents of the Amaryllidaceae, such as narciclasine, pancratistatin and their congeners, are the most

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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 sixty genera, whose eight hundred species are widely distributed in several countries in 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 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 Polynesia 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 in time because of their effective antitumoral and antiviral activities. Lycorine is a pyrrolo[*de*]phenathridine 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 Figure 1, Figure 2, Figure 3 and Figure 7.[22] The advances made in the isolation as well as chemical and biological characterization 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-ethanophenathridine groups due to reduced toxicity.[29] Narwedine (**4**, Figure 1) potentiates the pharmacological effects of caffeine, carbazole, arecoline, and to a lesser extent of nicotine, in laboratory animals. In addition, narwedine and vittatine (**12**, Figure 2) potentiate the analgesic effects of sub-optimal 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 dose. 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 is 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]

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 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 aeruginosae*. Furthemore, 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. *A. coronica*, a species which 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 *Bophane* genus are known for their large bulbs and production of characteristic alkaloids. *B. disticha* (L.f.) Herb. is known as a toxic plant, containing compounds with alleged hallucinogenic potential. However, bulb scales or infusion 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 activity. The bulbs are applied as antiseptic dressings on fresh wounds, whereas bulb decoctions are administered for the treatment of coughs, colds as well as abdominal, renal and liver complaints.[18]

Clivia species, namely *C. miniata* and *C. nobilis* are cultivated in Egypt as ornamental plants for their beautiful flowers. Root infusion of *C. miniata* (Lindl.) Regel is used to treat snakebites 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-dihydrolactone[2]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] as well as nobilisitine A and B[37] (**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 *Pseudomonas aeruginosa*) bacterial strains as well as and fungi (*C. albicans*). All alkaloids with the exception of clivimine showed antibacterial activity against Gram-positive *S. aureus*. The same compounds, in particular, nobilisitine 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 *C. zeylanicum* was used in the Moluccas as a strong poison.[38] *C. asiaticum* is known throughout southeast Asia and Polynesia as an emetic and diaphoretic, while *C. bulbispermum* is a general medicinal herb. In southern Sotho different parts of this plant are used to treat colds, 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 as a rubefacient in the treatment of rheumatism. *C. delagoense* plants are used in Zhulu and Xhosa traditional medicine to treat urinary problems, while in Australia, the aborigines obtain starch from *C. flaccidum* to eat as a kind of gruel. *C. giganteum* is a Congo leprosy remedy. *C. 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 *C. kirkii* are used as a purgative and the outer bulb scales as a rat poison. *C. 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. *C. moorei* bulbs in a decoction are used for swelling and urinary problems, while *C. pratense* is used in Indian medicine for intestinal diseases such as diarrhea and dysentery. [38] *C. 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, e.g. 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 *C. latofolium* and characterized on the basis of comprehensive spectral analysis, chemical transformations and synthesis.[39] The bulbs of *C. jagus* and *C. glaucum* are used in traditional medicine in southern Nigeria for memory loss and other mental symptoms associated with ageing. 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 activity 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 crinamine (**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 treatment of weight loss, low milk production, milk loss or for healthy calves and placenta retention in the 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 5,10b-ethanophenanthridine (e.g. vittatine or crinine, **12**, Figure 2 and **29**, Figure 6). Various trivial

names of *Crinum* alkaloids, their distribution 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. mackenii* 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]

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]

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]

Narcissus 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[5,23,24,25] 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 lycorine (**3**, Figure 1), homolycorine, 9-*O*-demethylhomolycorine and tazettine (**26**, **27** and **28**, Figure 5).[43,44]

The alkaloids isolated from *N. tazzetta* 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]

Pancratium genus has attracted considerable attention due to the complex structural types of its alkaloids[25] 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 trispheridirine[47] and then haemanthamine, pseudolycorine and 11-hydroxyvittatine.[48,49] Successively, from the same plant were isolated two 2-oxyphenathridinium betaine-type alkaloids: ungeremine and zefbetaine[50] (**34** and **36**, Figure 7), known for their very interesting biological activities (i.e. cytotoxic, antibiotic[51−53] and plant growth-regulatory[51]). 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-dydrolycorine (**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 phenanthridinuim derivatives were isolated and characterized as anhydrolycorinium chloride (**42**, Figure 7) and the dihydroderivative 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 *Zephyrantus*, *Z. parulla* Killip appears in the history of Peru (by Padre Cobo, 1653) for treating tumors, and *Z. Rosea* (Spreng.) Lindll. 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 rainy season (July–August). The extract of its bulbs is used in the indigenous system of medicine for a variety of therapeutic purposes, e.g. 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, pseudolycorine-1-*O*-β-D-glucoside, and four phenanthridinuim 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, though 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, immuno-stimulatory, 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. Since the biochemical content and pharmacological action of many of these species still remains 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* bulbs[63] in a systematic program aimed at the detection of antigrowth factors in plants, showed potent antimitotic activity.[64] The structure of narciclasine was then revised by a chemical study[65] 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 study[65] and found to be identical to arolycoricidinol obtained by acid treatment lycoricidinol.[63,67] Later, narciprimine and narciclasine were also isolated together from *Zephyranthes tubispatha*[68] and *Lycoris sanguinea*.[69]

Promising activity of narciclasine has stimulated much effort toward the isolation and chemical characterization of 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 *Pancratium 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-ᴅ-glucoside of pancratistatin (named pancratiside) (**52** and **53**, Figure 9).[71] Two other congeners of narciclasine, *trans*-dihydronarciclasine and its 7-deoxy derivative (**49** and **50**, Figure 9) were isolated correspondingly from *Z. candida*[74] and *Hymenocallis littoralis*, *H. caribea*, *H. latifolia*.[72] Thus, it appears that all three subtypes of these phenanthridones, namely narciclasine, pancratistatin and *trans*-dihydronarciclasine 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 isocarbostyril and phenanthridinuim alkaloids from an insect.[75]

Finally, a number of glycosyl derivatives of narciclasine and pancratistatin were isolated from different Amaryllidaceae species. As mentioned above, pancratiside is isolated *H. kalbreyeri*,[71] kalbreclasine and 4-*O*-β-ᴅ-glucosylnarciclasine (**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-ᴅ-glucopyranosyl-pancratistatin (**54**, Figure 9), named telastaside, 1-*O*-(3-hydroxybutyryl)-pancratistatin and 1-*O*-(3-*O*-β-ᴅ-glucopyranosylbutyryl)-pancratistatin (**55** and **56**, Figure 9) were isolated from the smokey-grey moth *Polytela gloriosa* (**54**)[78] and *Z. carinata* (**55**, **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 isocarbostyril 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 isocarbostyril compounds. The established processes could be summarized as follows.

Method A—The fresh grounded 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]

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 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, 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]

Method C—The fresh plant material is macerated in aqueous methanol in a high-speed blender, filtered and the filtrate evaporated under reduced pressure to give a viscous brown slurry. This was 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 as well as phenanthridinium and glycosyl alkaloids, respectively.[51]

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 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 separates the methylene chloride phase. The aqueous phase is concentrated, centrifuged and the resulting clear solution is extracted with *n*-butanol. The *n*-butanol extract is concentrated and its aliquot is dissolved 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]

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 hours to allow the mucilage to leach out and then washed four times. The mucilage solutions are combined, lyophilized and the residual powder is extracted with 70% ethanol using a Soxhlet. The extract is concentrated, extracted with ethyl acetate and the corresponding crude extract is fractionated by a silica gel column yielding a crystalline isocarbostyril constituent.[81]

Method A was applied to extract narciclasine from thirty two 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 were found in march at the flowering stage, but only 100–120 mg/kg 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-deoxynarciclasine from *L. radiata* Herb.; the yields for each isocarbostyril 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] and *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 isocarbostyril derivative from an insect. An 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 isocarbostyrils) from *H. littoralis* was developed. The bulbs were subdivided and placed in six a 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 were concentrated to dryness and the residue 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 fractioned 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 the classical method A.[63]

Method F—An alternative improved method F in respect to all 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 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 Shiff 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 labelled precursors.[88–90] Lycorine is the precursor of hippeastrine (**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 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 non-heterocyclic 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 labelled [6,10-^{3}H$_2$; 12-^{14}C]noroxomaritidine (**65**) into **9** as well as **5** and **67**.[96] In addition, a possible link between the biosynthesis of haemanthamine, haemanthidine and that of ismine was demonstrated. Thus, using a doubly labelled [6*R*,10-^{3}H$_2$]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**). Since 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'-^{3}H$_2$; ^{14}C-*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 compared with the expected 50% retention shown by lycorine pointed to the derivation of this isocarbostyril 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 ^{3}H activity without picking up of deuterium from the solvent. Since 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 narciclasic aldehyde (**72**, Figure 14), which retains the C4a hydrogen, the removed ^{3}H atom was assigned to position C4.[25] The results support the derivation of lactam **44** from **59** along the *para-para* oxidative coupling pathway.

Further evidence was obtained by incorporation a *meta*-labeled [2',6'-^{3}H$_2$; ^{14}C-*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 ^{3}H loss, while norpluvine (**61**) showed *ca.* 15% tritium loss. The ^{3}H labels were assigned to positions C1 and C4a of narciclasine because narciclasic aldehyde (**72**) retained all the ^{3}H activity and narciprimine (**48**) formed with 50% ^{3}H loss. The latter lost all of the ^{3}H 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-^{3}H$_2$] noroxomaritidine (**65**) and racemic [3-^{3}H$_3$]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 narciclasic aldehyde (**72**). The obtained relative ^{3}H-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 which are able to form a methylendioxy 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-^{3}H$_2$]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'-^{3}H$_2$]-**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 60,000 bulbs at present. In 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*-dihydroderivative.[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 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 Ba(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 Krohn[105] studied hydrogenation of the double bond in **44** and found that along with expected *trans* and *cis* 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**, **79**, **80**, **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 excess of acetic anhydride in pyridine, it is stopped after 3 hours 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 non-selectively producing two isomeric secondary alcohols **86** and **76**. These can be easily separated and, therefore, this transformation allows for the preparation of C2-*epi*-narciclasine.

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 OsO_4 in the presence of hydroquinine 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 2:1 ratio (Scheme 4). The acetonide group in **90** is smoothly removed with a solution of sulfuric acid in H_2O/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 with $RuCl_3 \cdot 3H_2O$/$NaIO_4$. 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 coworkers investigated radical a 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 equivalents of Ac_2O 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 Bu_3SnH 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 isocarbostyril.

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 ten steps and overall yield of 3.6%.

Another important investigation resulting in the synthesis of useful analogues of the natural isocarbostyril 7-deoxynarciclasine (**45**) and 7-deoxy-*trans*-dihydronarciclasine (**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 isocarbostyrils 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, reduction of the amide functionality with LiAlH$_4$ 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 isocarbostyrils 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 potential of this isocarbostyril 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 antimitotic activity against the cells of sarcoma 180 in ascites form when administered subcutaneously in low dosages. Studies by a number of laboratories, most prominently by Spanish investigators at the Instituto de Biologia 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 [^{14}C]phenylalanine incorporation in yeast polysomes and rabbit reticulocyte (immature red blood cells) ribosomes.[110] The authors further went on to show that the isocarbostyril inhibited the association of both [^{3}H]anisomycin and [^{14}C]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* TR$_1$ 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 isocarbostyril inhibits the uptake of [^{3}H]leucine into the proteins of the wild type *Saccharomyces 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 seventeen Amaryllidaceae alkaloids on (a) HeLa cell growth and (b) DNA, RNA and protein syntheses in intact Krebs ascites cells.[113] DNA, RNA and protein syntheses were studied by incorporation of [^{14}C]thymidine, [^{14}C]uridine and [^{14}C]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 reaction conditions the electrophilic aromatic substitution takes place to give C10-labeled compound.

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

[^{3}H]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[^{14}C]phenylalanine synthesis by yeast ribosomes and the activity patterns were correlated with the ability of these compounds to inhibit the binding of [^{3}H]narciclasine to yeast ribosomes (Table 4).

Thus, *trans*-dihydronarciclasine (**49**), *trans*-dihydronarciclasine acetonide (**79**) and isonarciclasine (**78**) inhibit both polyphenylalanine synthesis and [^{3}H]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 [^{3}H]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 acetonide (**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"

The 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 ten cut roses (var. Baccara) were placed in a vase together with ten daffodils (var. Carlton) for 4 hours 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 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) were 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 isocarbostyril interferes with the elongation growth of *Avena* coleoptiles rice seedlings,[67] etiolated pea epicotyls,[120] cress roots,[121] and inhibits the callus growth of cultured tobacco piths[67] 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

$[^{14}C]$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) is 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 fussicoccin-induced growth is only partially inhibited. Since it is known that IAA effect on plant growth fully depends on protein synthesis and fussicoccin 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-deoxynarcilasine 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 forty 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 (kalbreclasine, **46**) and pancratistatin (pancratiside, **53**) are intriguing.[71] Thus, Ghosal *et al.* detailed such contrasting behavior for the pancratistatin/pancratiside pair in 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 hours, 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 phenomenon is general for these classes of Amaryllidaceae constituents, since similar observations were reported for lycorine/lycorine-1-*O*-β-glucoside pair.[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 antimitotic 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 hours of administration at the doses of 0.9 mg/kg. In addition, an LD_{50} value of 5 mg/kg in mice was established for the subcutaneous injection administration route. Working independently at a about the same time, Okamoto *et al.* reported toxic effects of narciclasine and 7-deoxynarciclasine toward the cells of Ehrlich Carcinoma, with the former isocarbostyril

being more potent.[67] Mondon and Krohn reported the first SAR investigation of narciclasine and its derivatives by assessing their toxicities to HeLa and HEP_H 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 µg/mL. Significantly less potent were *cis*-dihydronarciclasine (**77**) and *O*,*N*-dimethylnarciclasine, while narciclasine acetonide (**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[^{14}C]phenylalanine synthesis as well as inhibition of binding of [^{3}H]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 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 GI_{50} values as low as 0.01 µg/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 sixty human cancer cell line *in vitro* screen.[135] The GI_{50} values, as reported Pettit *et al.*,[72] are averaged across the entire sixty 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 isocarbostyrils (**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 isocarbostyrils does not show good correlation with any of the known anticancer agents. 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 coworkers concluded their analysis of NCI screening data with the following statement: "Indeed, it seems likely that pancratistatin and related *trans*-dihydrocarbostyrils, as well as narciclasine, constitute the most important antineoplastic components of Amaryllidaceae species used 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 LD_{50} value of 0.88 µg/mL for **47** (LD_{50} = 0.29 µg/mL was obtained for **44** in this assay). Both **44** and **47** exhibited antitumor activity in the potato disc 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 non-glucosylated (**55**) and glucosylated (**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 coworkers reported the results of investigations of the effects of pancratistatin on normal and cancer cells, providing evidence supporting selective toxicity of this isocarbostyril to cancer cells. Thus, pancratistatin was found to selectively induce apoptosis using non-genomic targets in 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 non-cancerous cell lines.[139] Finally, the authors speculated that pancratistatin may take advantage of the differences between mitochondria in cancerous and non-cancerous 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 coworkers have tested all of the synthetic intermediates shown in Scheme 3, Scheme 4, Scheme 5, Scheme 6 and Scheme 7, which represent a large selection of analogues accessible from the natural isocarbostyrils, 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 junction, 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 isocarbostyril by a factor of 10.[108] An intriguing discovery is 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 isocarbostyrils.[84]

Synthetic efforts by many research groups, aimed at preparation of analogues inaccessible from the natural isocarbostyrils, have been invaluable in defining the cytotoxic pharmacophore(s) associated with these compounds to a much greater extent. The analogues, prepared from commercially 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. Further, 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] and, 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 methylenedioxybenezene 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**, **50**, **51**, **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 coworkers 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, 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 coworkers.[137] Due to such profound differences in structure, it is likely that this compound works through a different mechanism than the parent isocarbostyril. Further, Chapleur and coworkers 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 coworkers 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, since 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 coworkers 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*-dihydronarcilasine (**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**, completely lacking any hydroxyl groups in ring C were prepared, but unfortunately not evaluated for biological activity.[152–154] Similarly, Banwell and coworkers 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**, **133**, **134** by McNulty and coworkers[156,157] as well as **135** by Hudlicky and coworkers,[141] have clearly indicated the importance of having at least three hydroxyl groups in ring C for potent cytotoxic activity. **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, while **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's[159] and Alonso's[160] groups respectively. However, no biological activity data were provided.

Finally, Keck's and Hudlicky's 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 haven't been disclosed yet, 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 pseudo-enantiomeric 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 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*-dihydronarciclasine is 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 US 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 effect against sandfly fever virus. Among the analogue

series, antiviral activity (expressed as IC_{50}) generally closely parallels toxicity to uninfected cells (expressed as TC_{50}) producing therapeutic indices (TI) values ranging between 3 and 8. Since 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 isocarbostyril was evaluated against *Escherichia coli HpR*.[64] The GI_{50} value of 8 µg/mL was established. These data are intriguing given the fact that narciclsaine does not inhibit protein biosynthesis in *E. coli*,[110] nor does it bind to *E. coli* ribosomes, as was established using experiments with [^{3}H]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 isocarbostyrils were reported to inhibit the *in vitro* proliferation of *Encephalitozoon intestinalis*, a microsporidian, which causes intestinal and systemic infections in immunocompromised patients. The IC_{50} values are 0.18 and 0.2 µM, eight times lower than the corresponding IC_{50} 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 (pancratiside, **53**) have unusual effects on plant physiology stimulating growth as opposed to the growth-inhibitory action of the non-glycosylated isocarbostyrils. 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 concentration 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 isocarbostyril's powerful anti-metastatic 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 isocarbostyrils 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 isocarbostyrils susceptible to the action of human non-specific 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-Pr$_2$NP(OBn)$_2$ and its subsequent oxidation to the corresponding phosphate proved to be irreproducible due to the instability of the phosphorous reagent.[174] An improvement of this method involves the reaction of **142** with dibenzylphosphite and carbon tetrachloride in presence of i-Pr$_2$NEt 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 mutistep 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 isocarbostyrils was found. The method involves reacting the corresponding isocarbostyril with tetrabutylammonium dihydrogenphosphate and dicyclohexylcarbodiimide in pyridine 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 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 isocarbostyrils, 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 antivascular/antiangiogenesis mechanism for the *in vivo* 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 isocarbostyrils 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 coworkers developed a biotechnological route to pancratistatin, narciclasine, 7-deoxynarciclasine and 7-deoxy-*trans*-dihydronarciclasine involving an initial tissue culture cloning of *H. littoralis* bulbs.[83,104] Although this effort led to the isolation of ca. 15 grams of pancratistatin and 12 grams of 7-deoxynarciclasine, these quantities are only sufficient to satisfy an interim demand for these agents. In general, due to the low natural abundance of the Amaryllidaceae anticancer isocarbostyrils (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 of 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] and, 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 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 coworkers.[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 Coworkers

The chemoenzymatic approach to the Amaryllidaceae anticancer isocarbostyrils by Hudlicky and coworkers 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 grams, 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-Ader 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)phenyliodinane 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$_N$2 fashion (as opposed to S$_N$2') 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 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 **157β** retains the TBS ether group after treatment with Na/anthracene, while the minor **157α** is obtained with the free phenolic hydroxyl. Therefore, **157β** is further desilylated to converge on the **157α** 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 debenzylation 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's[189] and later Keck's[162] groups have shown this efficient way of construction of such regiochemically complex benzene derivativatives, 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 MnO$_2$ in the presence of Me$_2$NH. 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 coworkers 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, e. g. 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 isocarbostyril **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 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 CF_3CO_2H gives the target isocarbostyril **45**.

This pathway constitutes the shortest synthesis of (+)-7-deoxynarciclasine (8 steps from diol **151**, 9 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.

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

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

Readily available D-gulonolactone (**179**, 79 USD / 25 grams, Aldrich) is converted to diacetonide 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 isocarbostyrils. 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 highly regioselectively 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 reprotection of the phenol as methyl ether are followed by the treatment with Me$_3$Al 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 coworkers 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 isocarbostyril by rearrangement to the corresponding lactam (Figure 24).

Unfortunately, such 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 affords alcohol **195**. Oxidation of the latter to the corresponding aldehyde is followed by its conversion to the desired N-aziridinylimine **196**. The key radical cascade cyclization event proceeds cleanly with the use of Ph$_3$SnH to give **197** as a single diastereomer in excellent yield for such a complex transformation. The completion of the synthesis involves N–O

bond cleavage with SmI_2, 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 $BF_3 \cdot Et_2O$ in CH_2Cl_2 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 isocarbostyril **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 isocarbostyrils, 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 Coworkers

Yan and coworkers described an efficient synthesis of (+)-narciclasine[186] 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).

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 the chloronitroso compound **200** with diene **201**, followed by the reductive N–O cleavage with Al-Hg (Scheme 18). **200** is derived from ketopinic acid (**199**, 65 USD/1 gram, 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 non-regioselective 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** → **212**).

9.4 Synthesis of (+)-Pancratistatin by Li and Coworkers

Li and coworkers 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 grams, Aldrich) represents somewhat expensive, but

attractive starting material as it already incorporates four stereogenic centers found in (+)-pancratistatin.

Selective protection of the *trans* (**215 → 216**) and *cis* (**216 → 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 $MgBr_2 \cdot OEt_2$ 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 $CeCl_3$ leads to the desired transformation via the presumed arylcerium intermediate. Finally, complete deprotection is accomplished by treating **224** with BBr_3 in CH_2Cl_2, 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 form 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 Hippokrates in the forth 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 non-basic 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 implementation 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. Since 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 isocarbostyrils.

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 isocarbostyrils. The mechanistic studies, revealing inhibition of protein biosynthesis by narciclasine and some of its natural and synthetic congeners, as discussed in this review, are thirty 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 isocarbostyrils 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 and, 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 coworkers 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.

Acknowledgments

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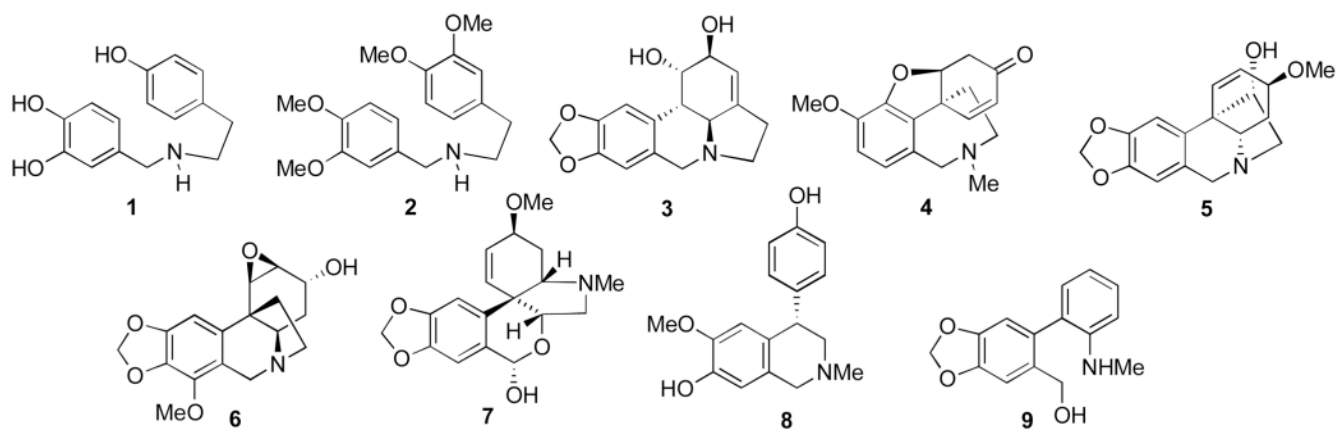
Figure 1.
Ring types and representative Amaryllidaceae alkaloids (see Table 1).
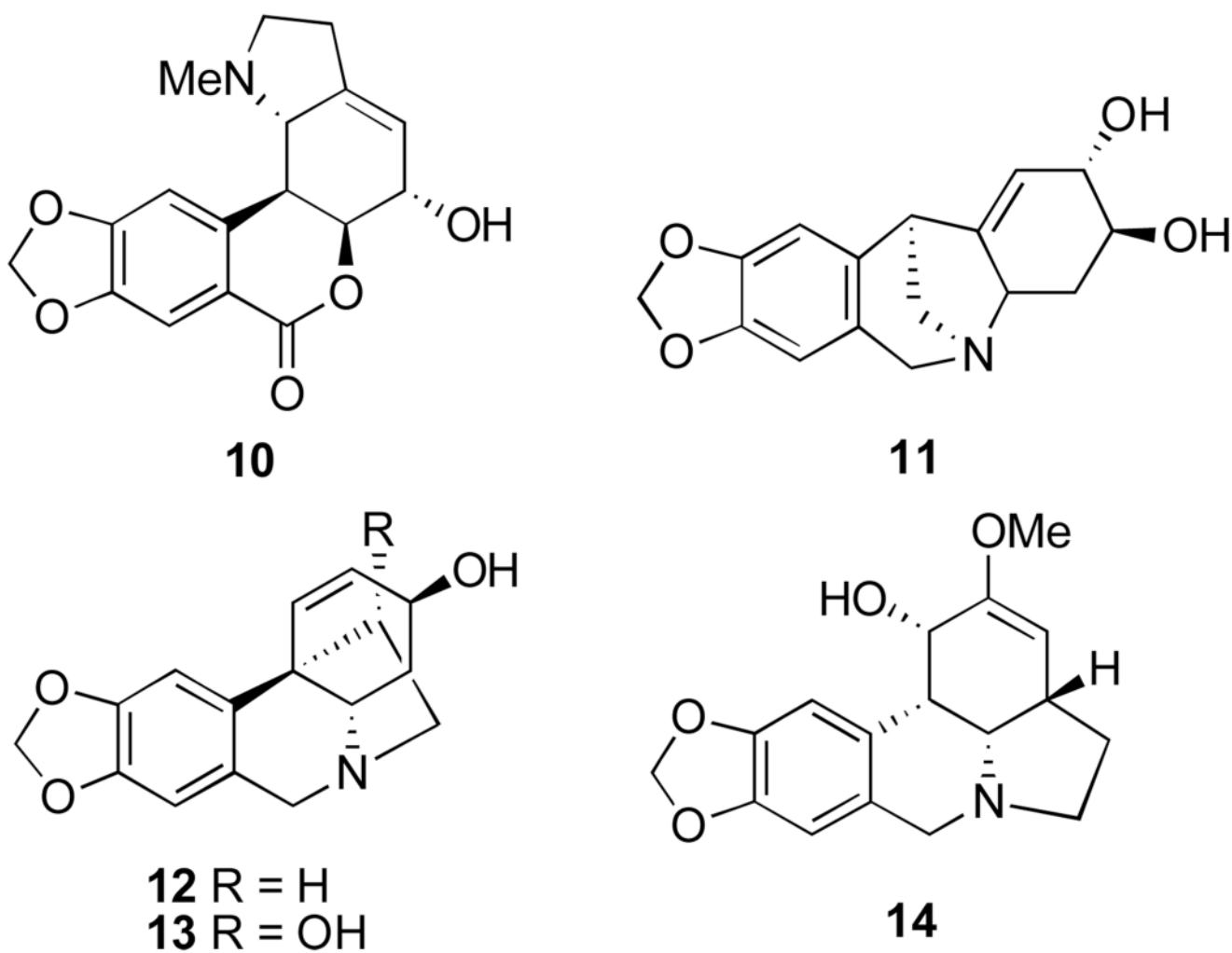
Figure 2.
Alkaloids from *Amaryllis belladonna* L.: hippeastrine (**10**), pancracine (**11**), vittatine (**12**), 11-hydroxyvittatine (**13**) and amarbellisine (**14**).
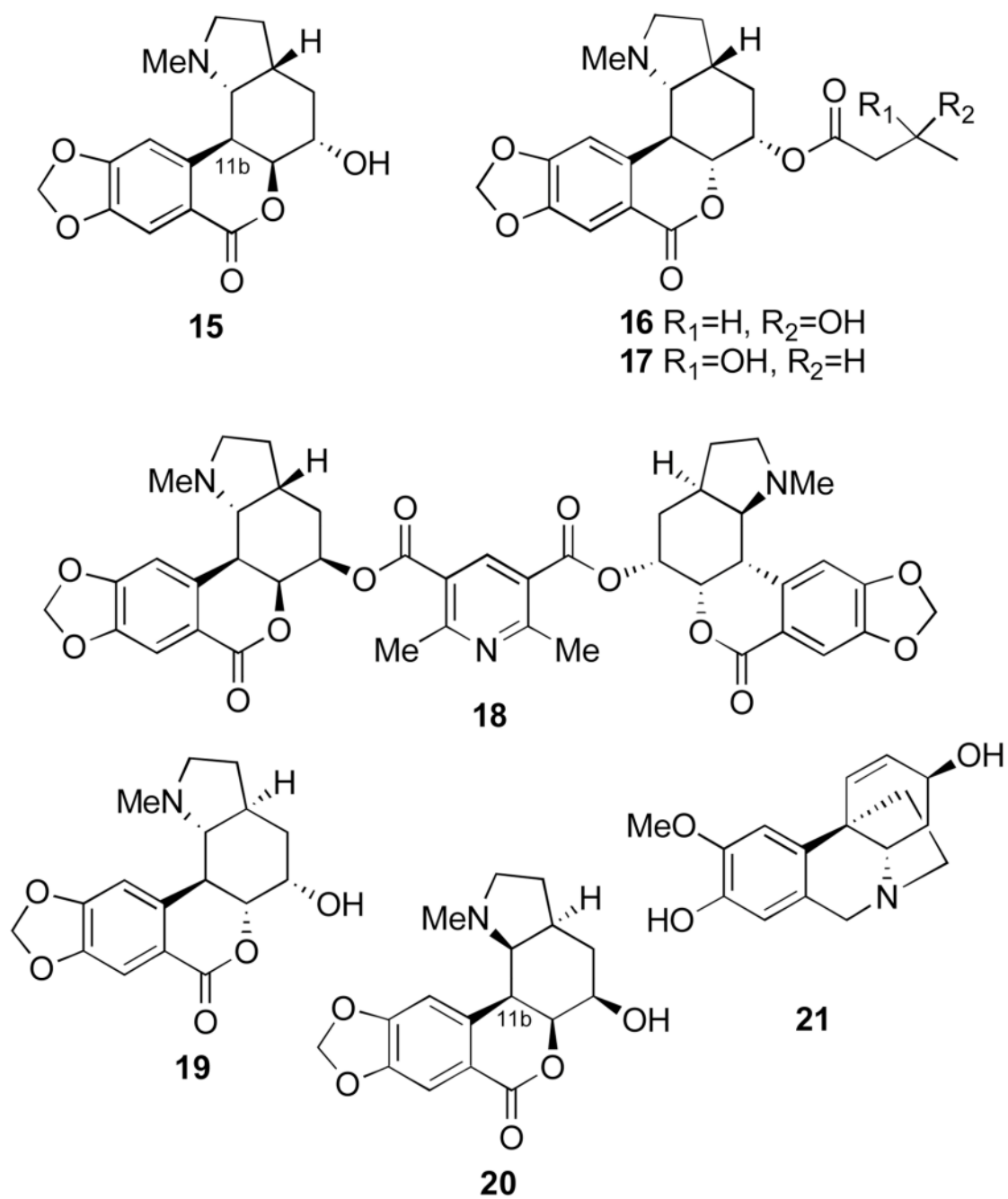
Figure 3.
Alkaloids from *Clivia* spp.: clivonine (**15**), clivatine (**16**), clivimine (**18**), nobilisine (**19**), nobilisitine A (**20**) and B (**17**), and (+)-8-demethylmaritidine (**21**).
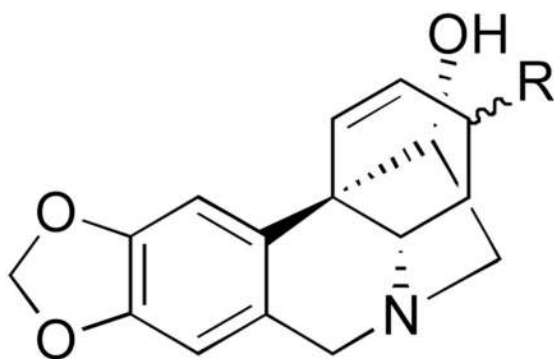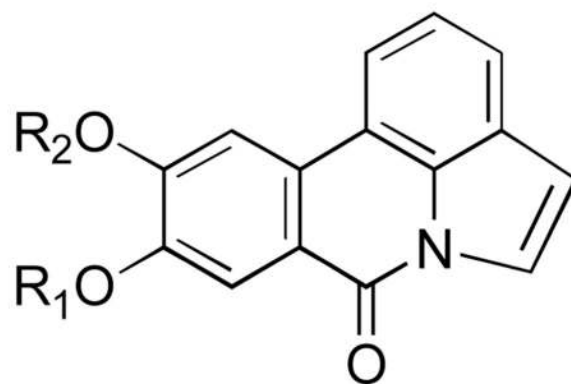
Figure 4.
Selected alkaloids from *Crinum* spp.: crinamine (**22**), hyamine (**23**), pratorimine (**24**) and pratosine (**25**).
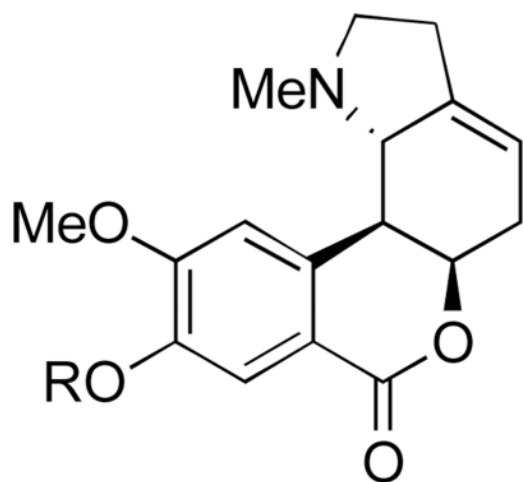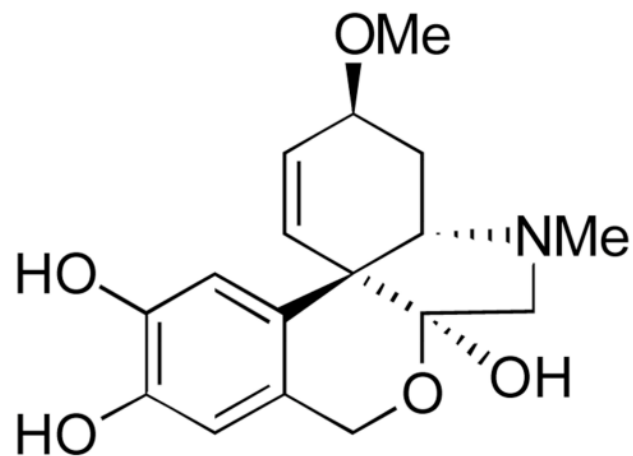
Figure 5.
Alkaloids from *Narcissus tazetta* L.: homolycorine (**26**), its 9-*O*-demethyl derivative (**27**) and tazettine (**28**).

29

30 R_1=H, R_2=H

31 R_1=Me, R_2=α-OH

32

33
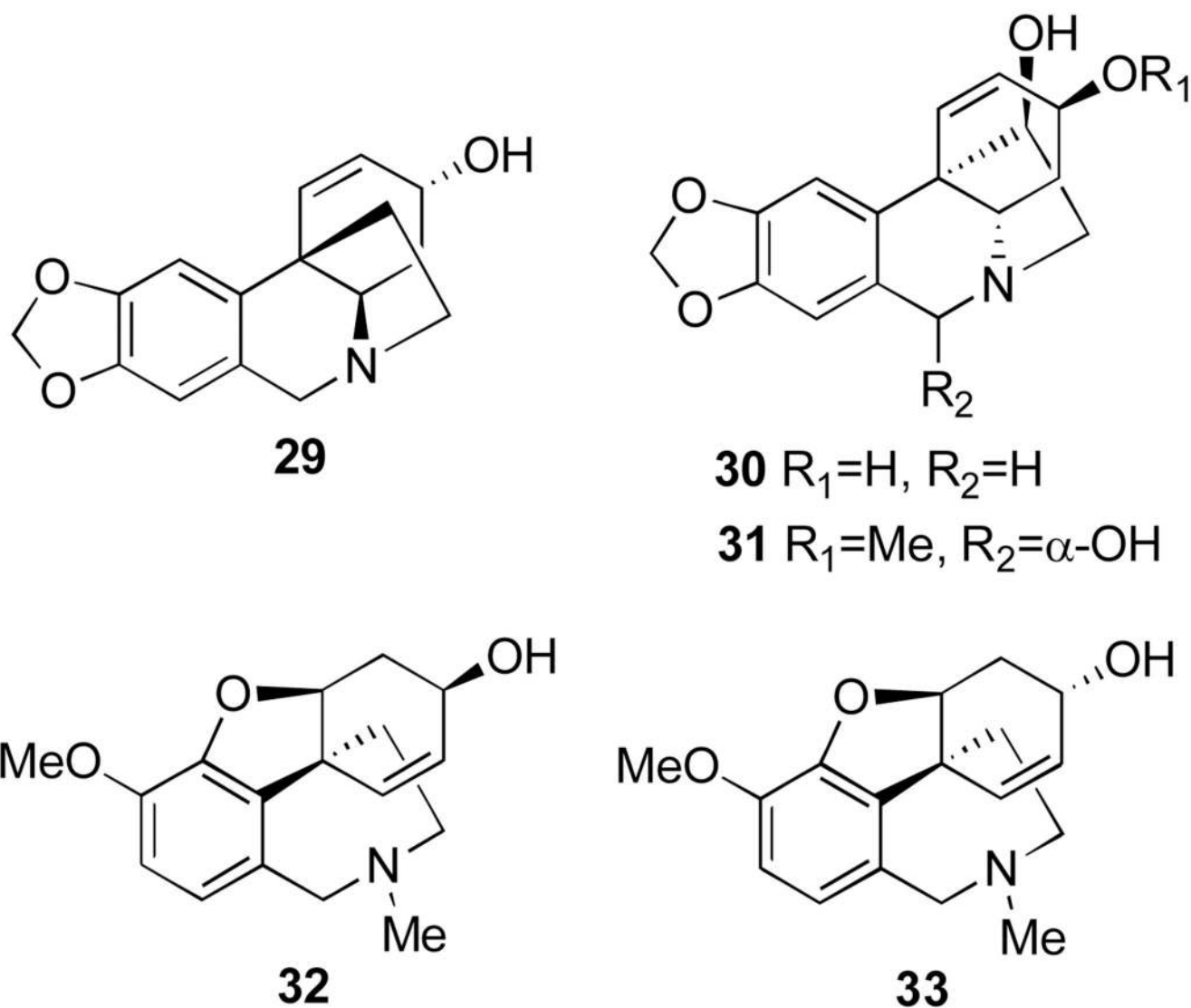
Figure 6.
Alkaloids from *Leucojum aestivum*, *N. tazetta* ssp. *tazetta* and *Pancratium maritimum* exhibiting antimalarial activity: crinine (**29**), 3-*epi*-hydroxybulbospermine (**30**), 6-hydroxyhaemanthamine (**31**), galanthamine (**32**) and 3-*epi*-galanthamine (**33**).
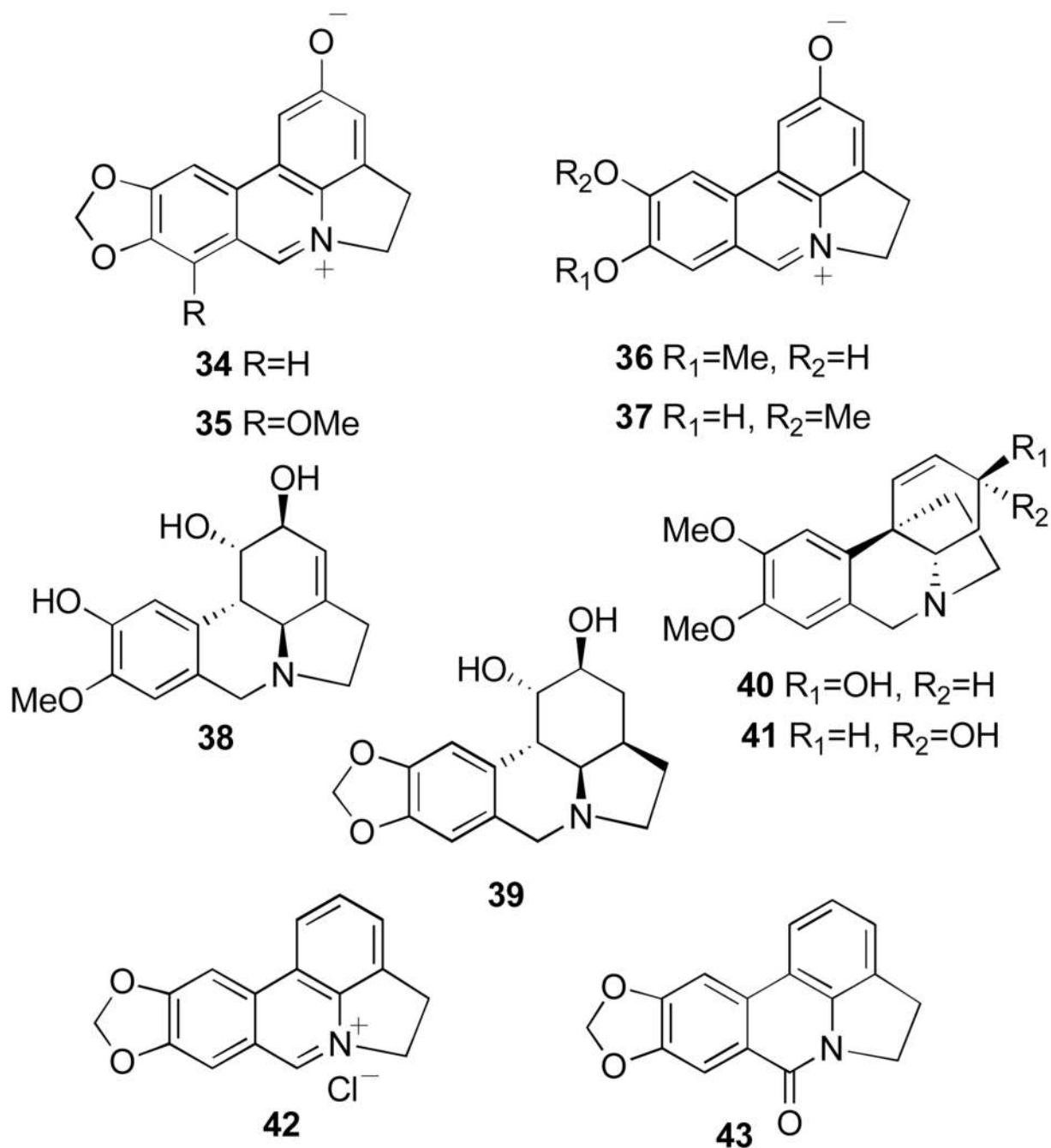
Figure 7.
Phenanthridiunium betaine alkaloids from *Pancratium* and *Zephyranthes*: ungeremine (**34**), zeflabetaine (**35**), zefbetaine (**36**), *iso*-zefbetaine (**37**), pseudolycorine (**38**), α-dihydrolycorine (**39**), maritidine (**40**) and (+)-*epi*-maritidine (**41**), as well as lycorine oxidation derivatives anhydrolycorinium chloride (**42**) and anhydrolycorine lactam (=crinasiadine, **43**).

Sternbergia lutea

Pancratium maritimum

Narcissus tazetta

Clivia nobilis

Figure 8.
Representative amaryllidaceae plants used in traditional medicine. Reprinted with with permission of Springer Science and Business Media (Reference 28).
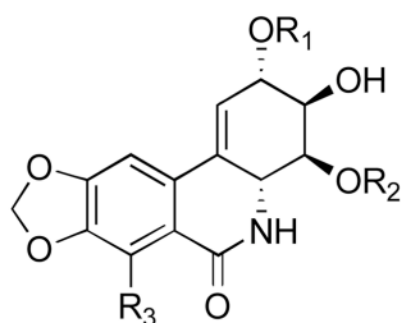
44 R$_1$=R$_2$=H, R$_3$=OH

45 R$_1$=R$_2$=R$_3$=H

46 R$_1$=β-D-Glc*p*, R$_2$=H, R$_3$=OH

47 R$_1$=H, R$_2$=β-D-Glc*p*, R$_3$=OH
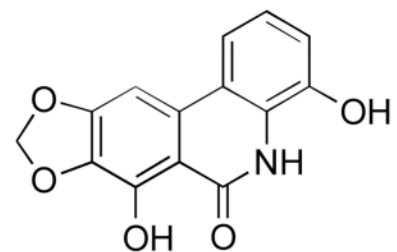
48
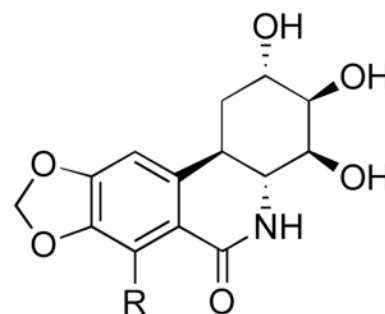
49 R=OH

50 R=H
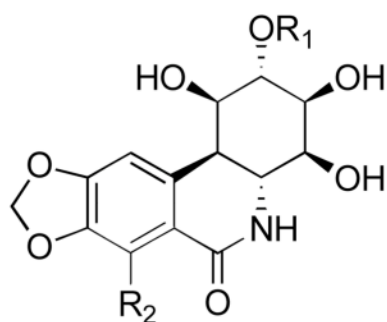
51 R$_1$=H, R$_2$=OH

52 R$_1$=R$_2$=H

53 R$_1$=β-D-Glc*p*, R$_2$=OH

54 R$_1$=(3-deoxy-3-*N*-Ac)-β-D-Glc*p*, R$_2$=OH
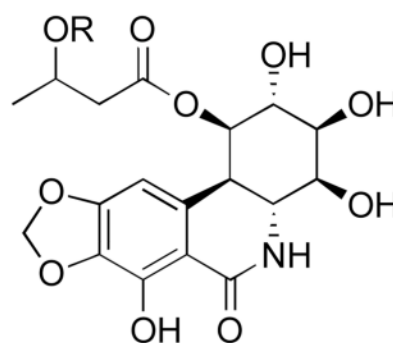
55 R=H

56 R=β-D-Glc*p*

Figure 9.
Narciclasine (**44**), pancratistatin (**51**) and their corresponding naturally occurring analogues
(**45–50**) and (**52–56**).
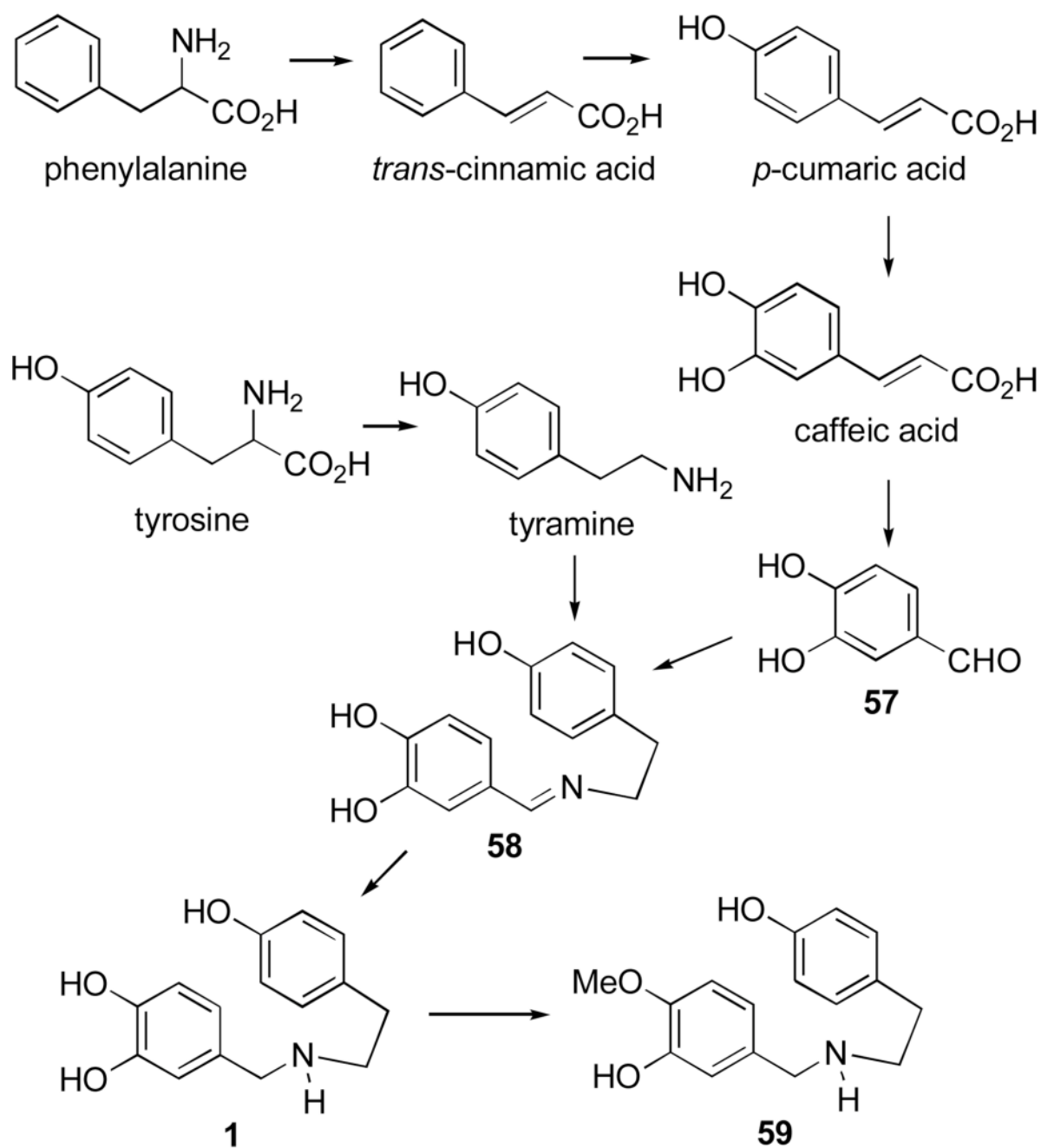
Figure 10.
Biosynthesis of *O*-methylnorbelladine (**59**).
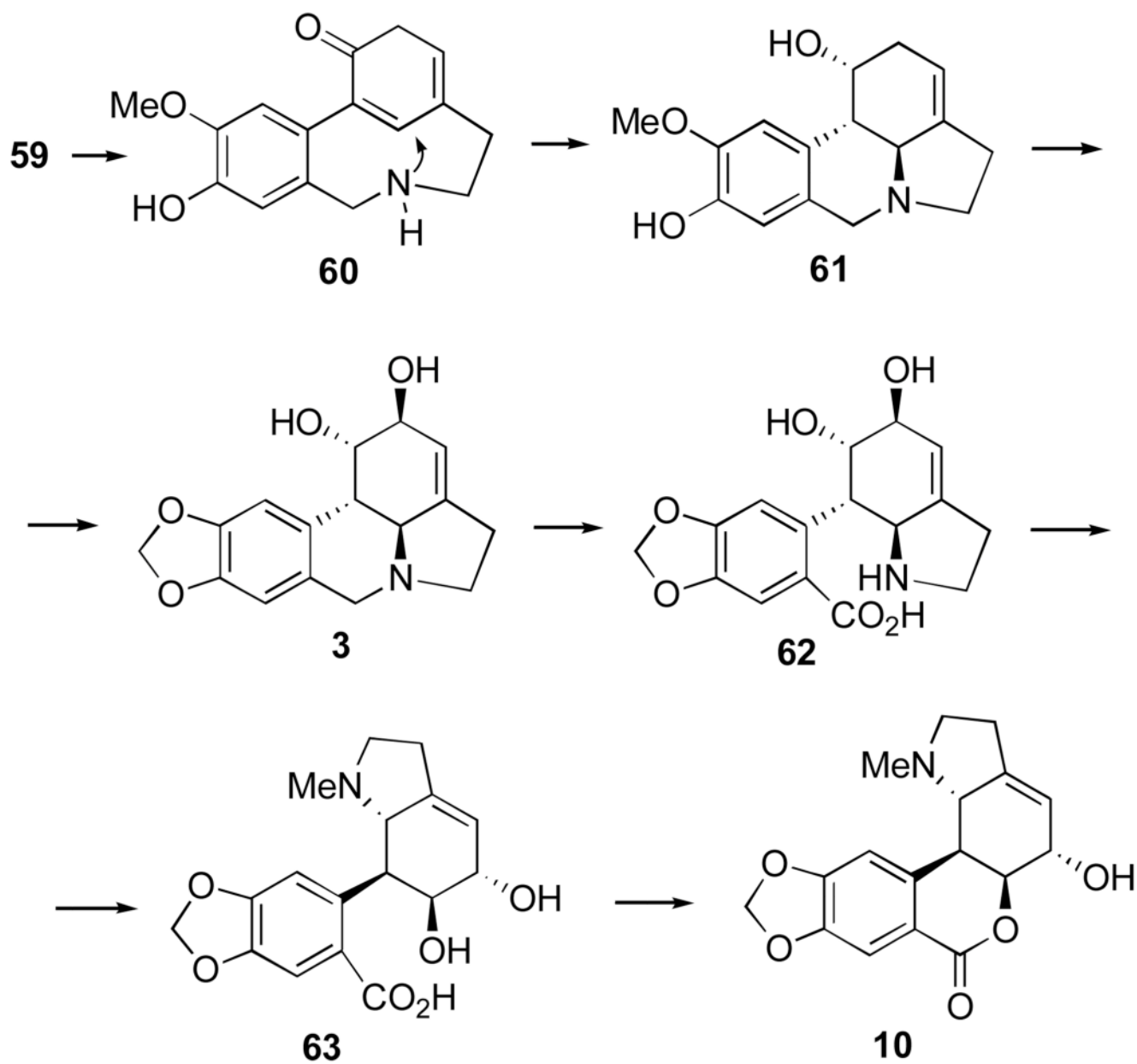
Figure 11.
Biosynthesis of Amaryllidaceae alkaloids by *para-ortho* oxidative coupling of *O*-methylnorbelladine.
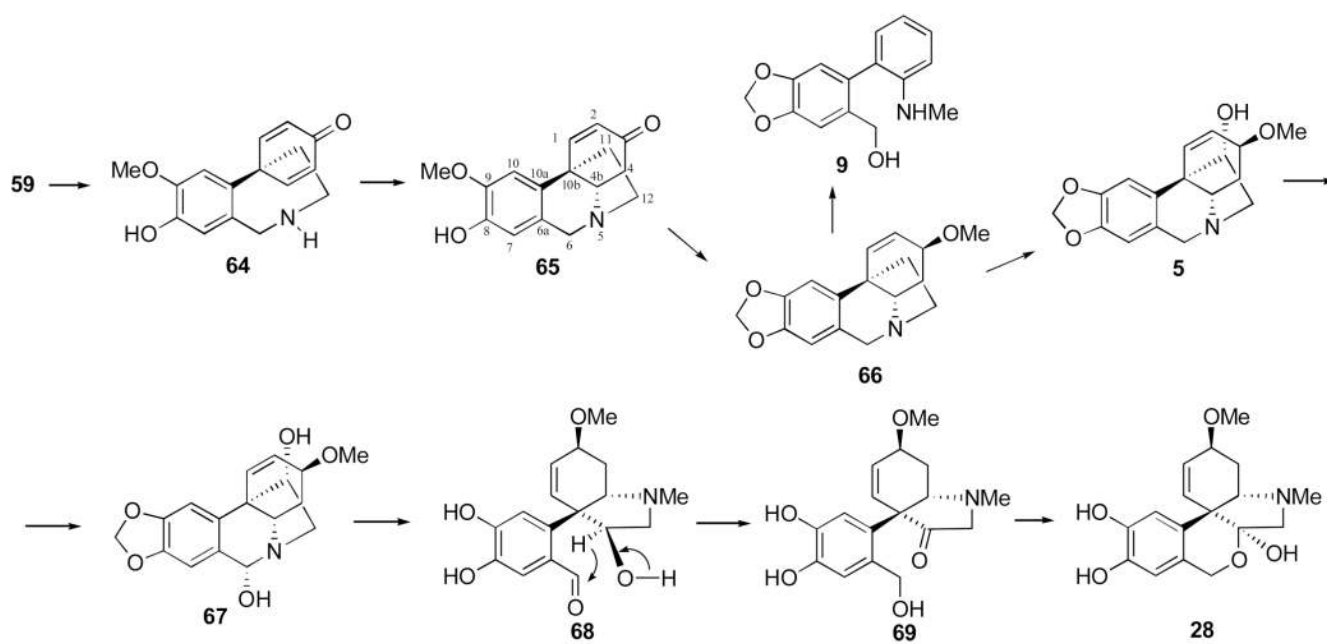
Figure 12.
Biosynthesis of Amaryllidaceae alkaloids by *para-para* oxidative coupling of *O*-methylnorbelladine.
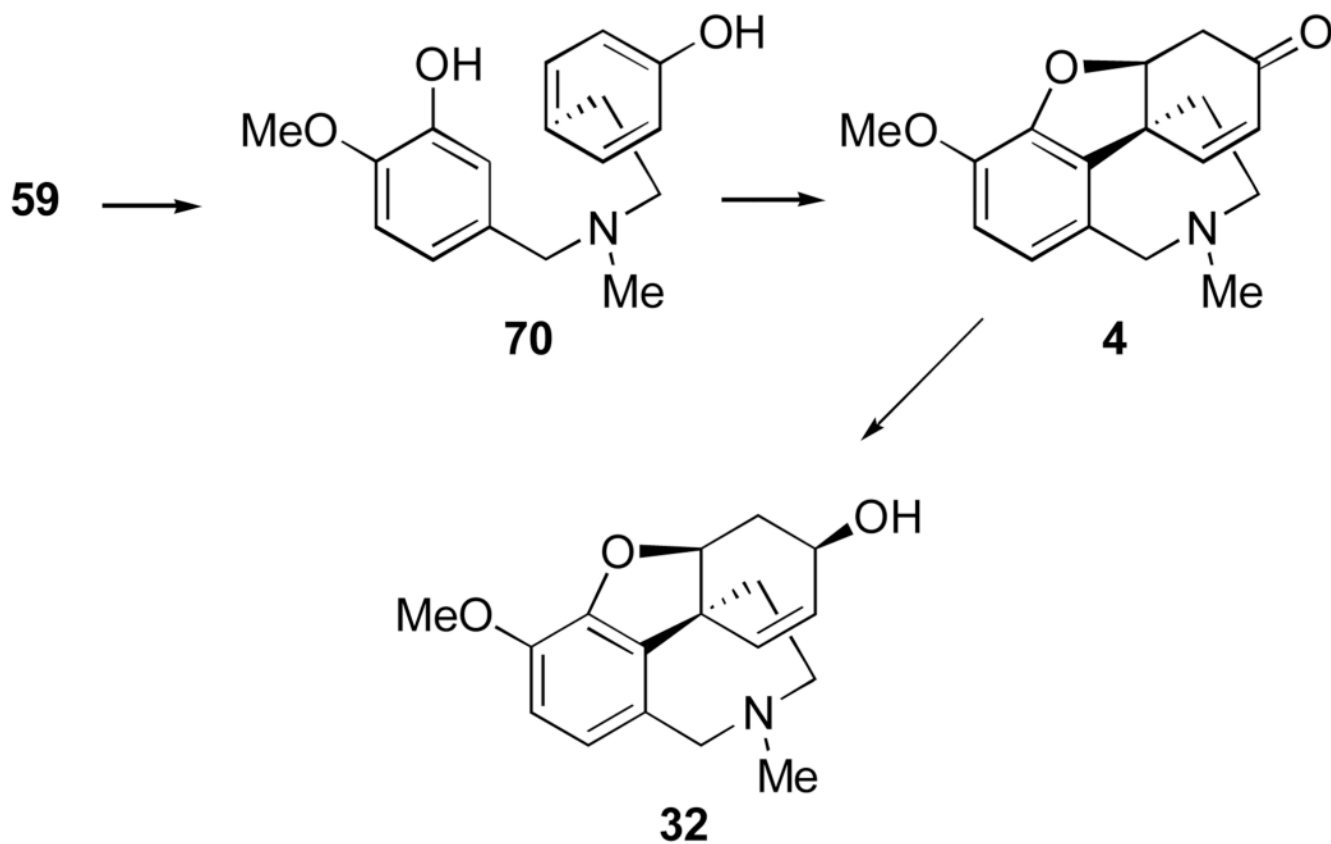
Figure 13.
Biosynthesis of Amaryllidaceae alkaloids by a third mode of oxidative coupling of *O*-methylnorbelladine.
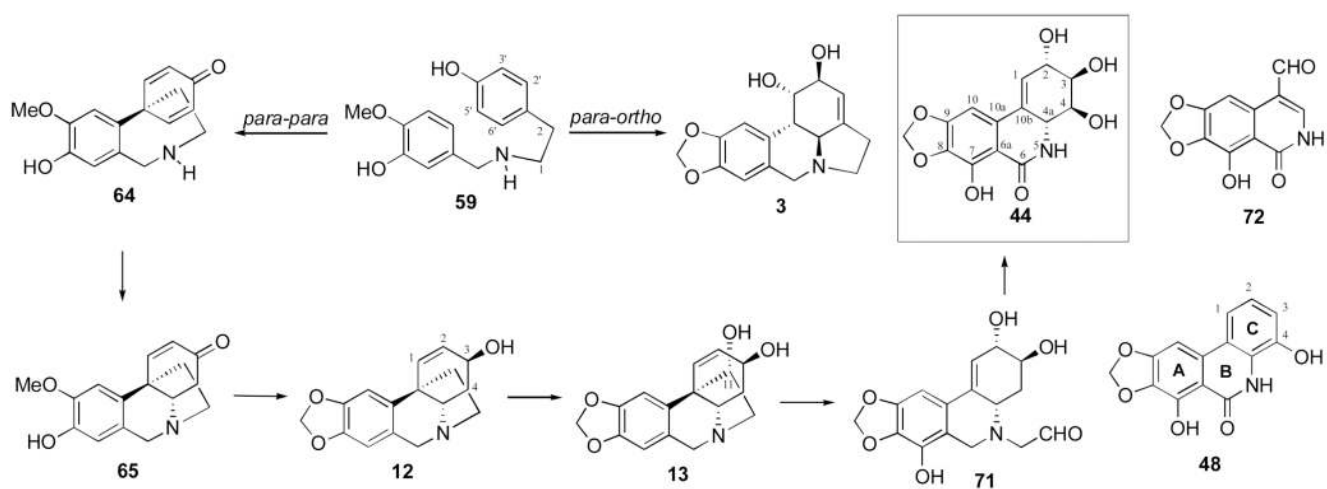
Figure 14.
Biosynthesis of narciclasine (**44**).
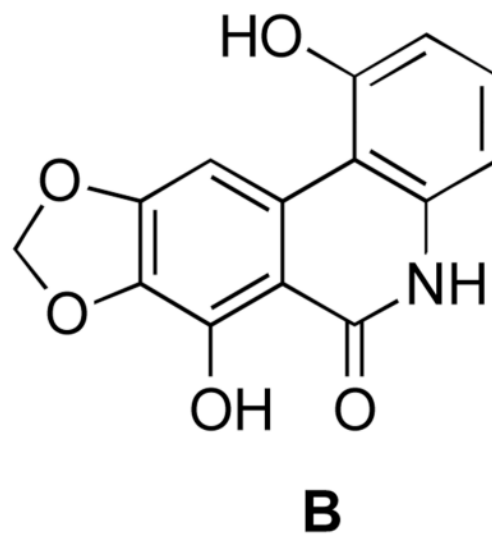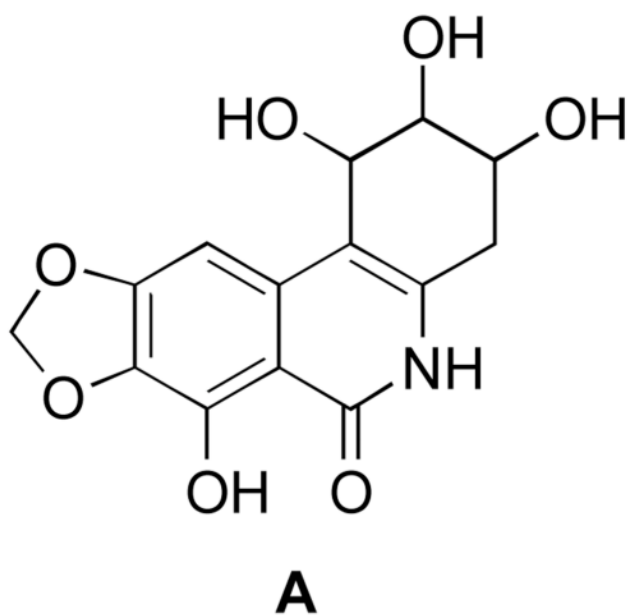
Figure 15.
Incorrect original structure assignment of narciclasine (**A**) and narciprimine (**B**).
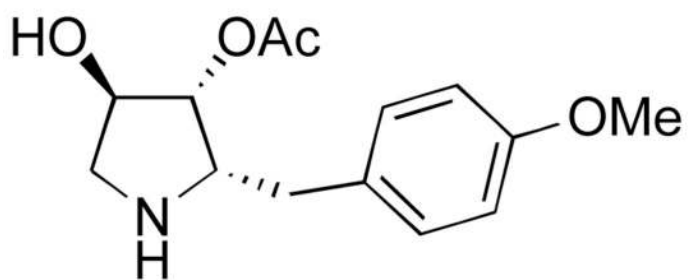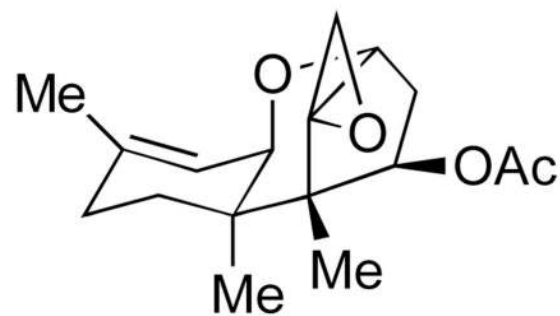
Figure 16.
Structures of anisomycin and trichodermin.
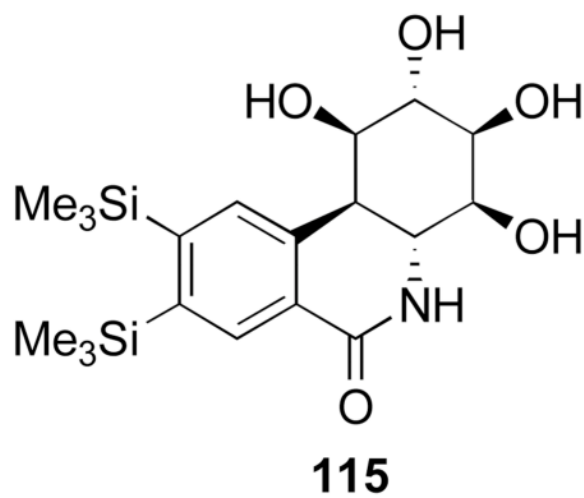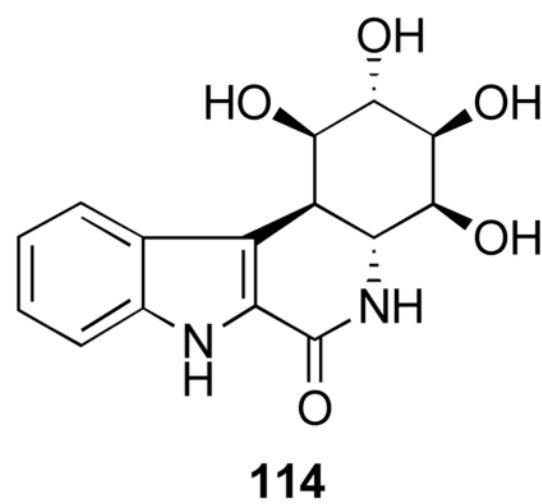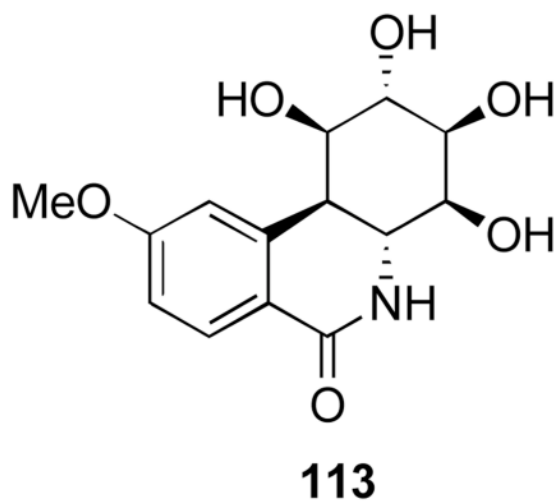
Figure 17.
Ring A analogues.
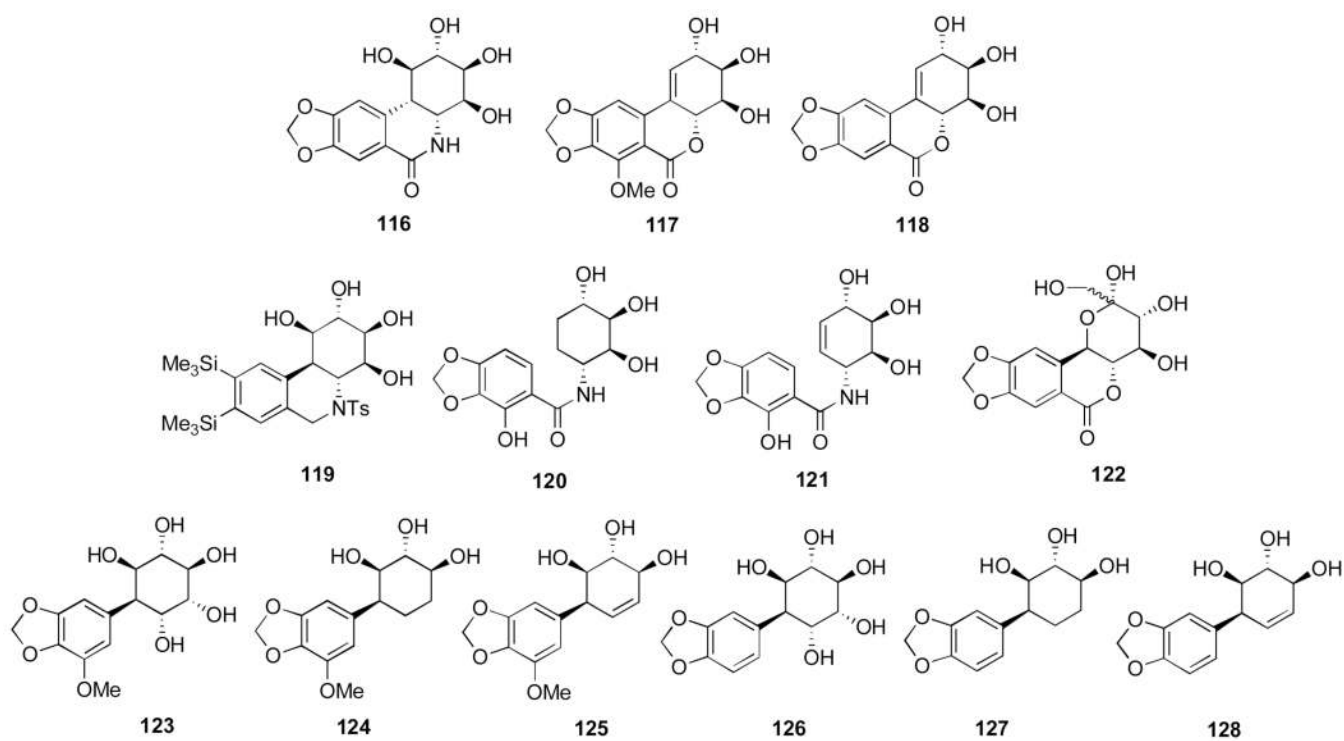
Figure 18.
Ring B analogues.
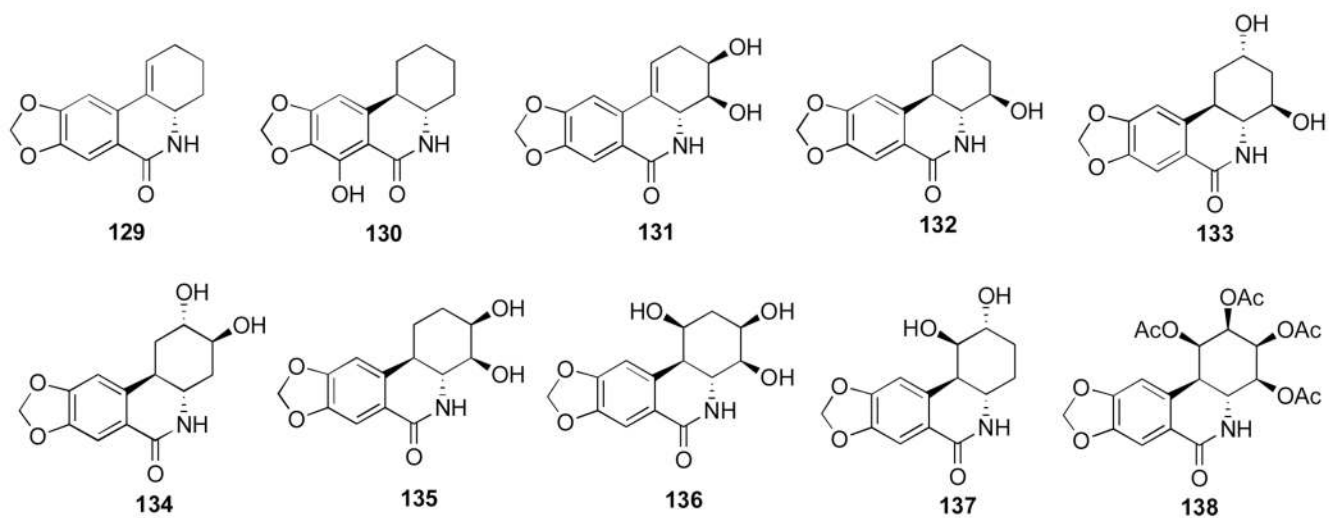
Figure 19.
Ring C analogues.
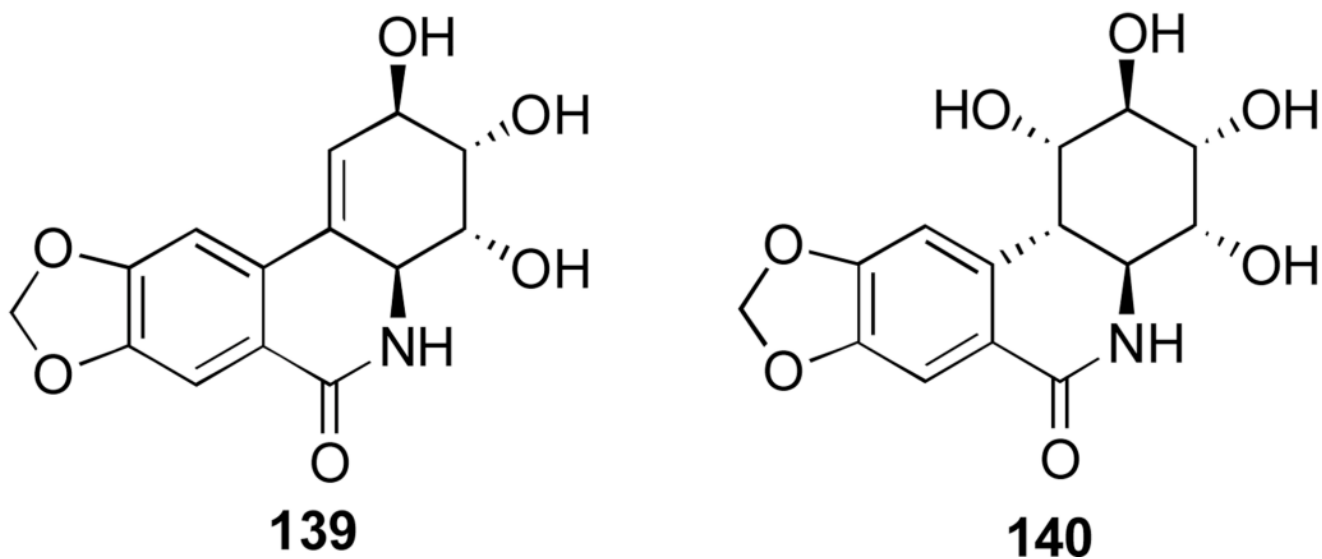
Figure 20.
Structures of *ent*-7-deoxynarciclasine and *ent*-7-deoxypancratistatin.
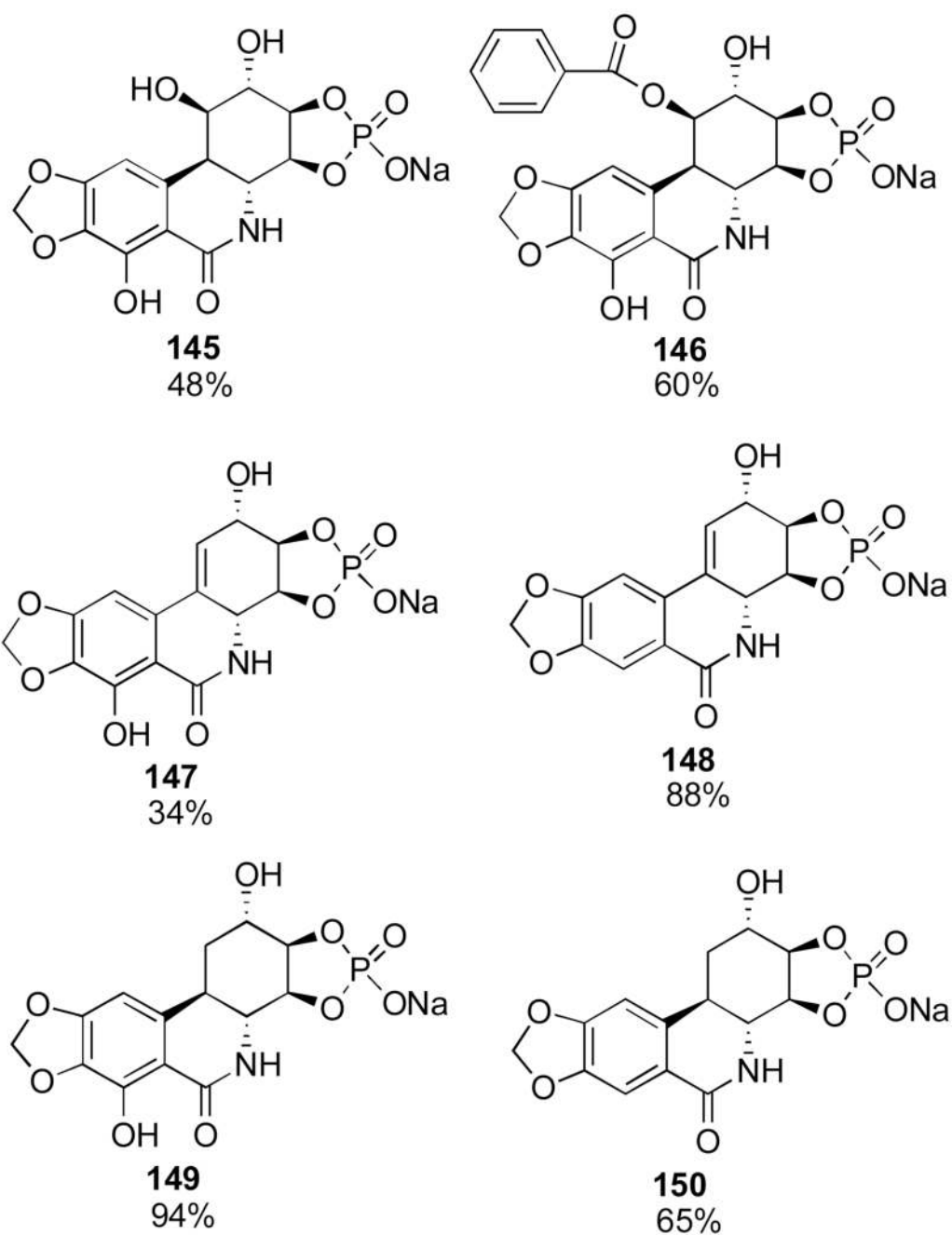
Figure 21.
Structures of 3,4-cyclic phosphate prodrugs.
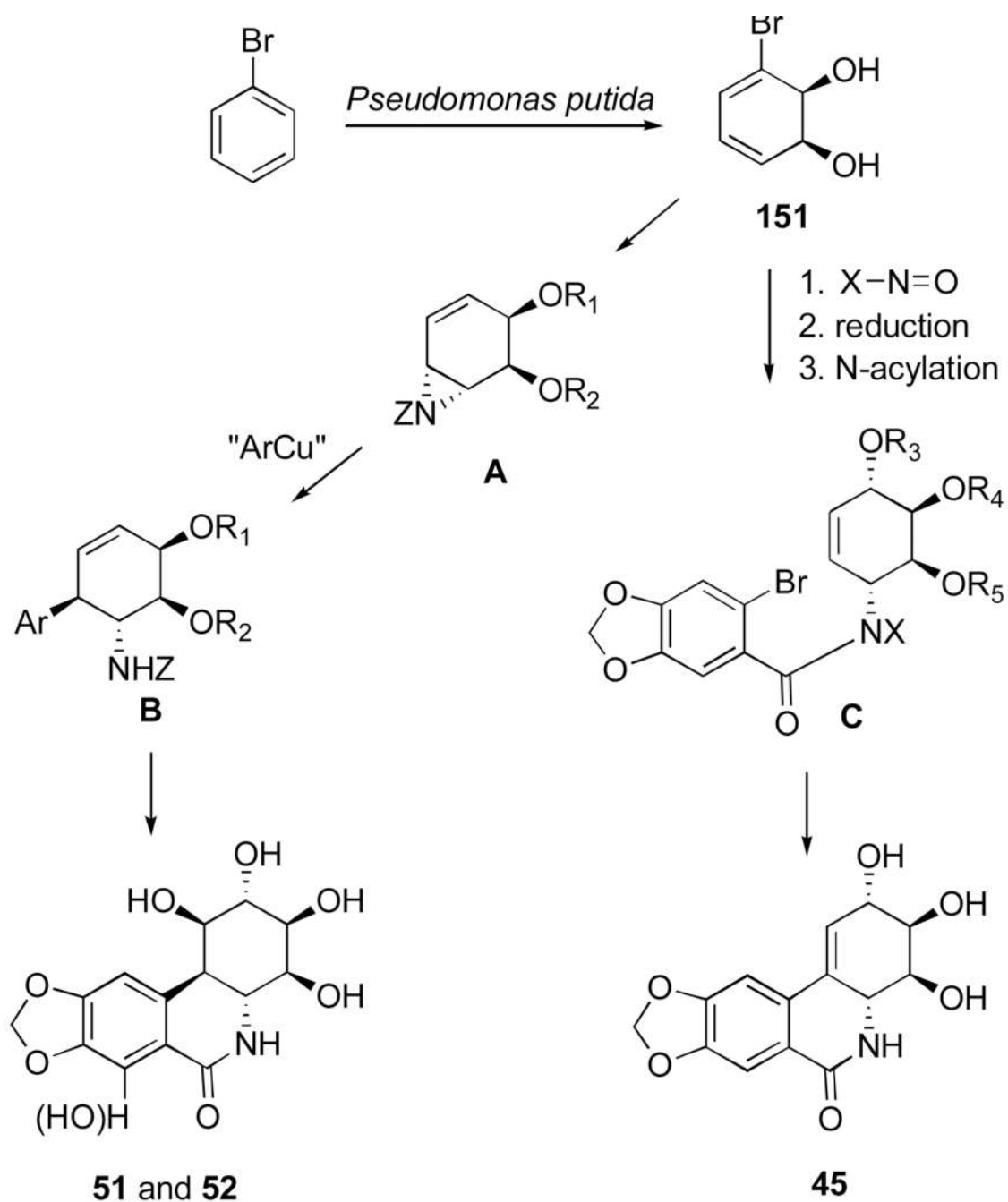
Figure 22.
Chemoenzymatic strategy to (+)-pancratistatin, (+)-7-deoxypancratistatin and (+)-7-deoxynarciclasine.
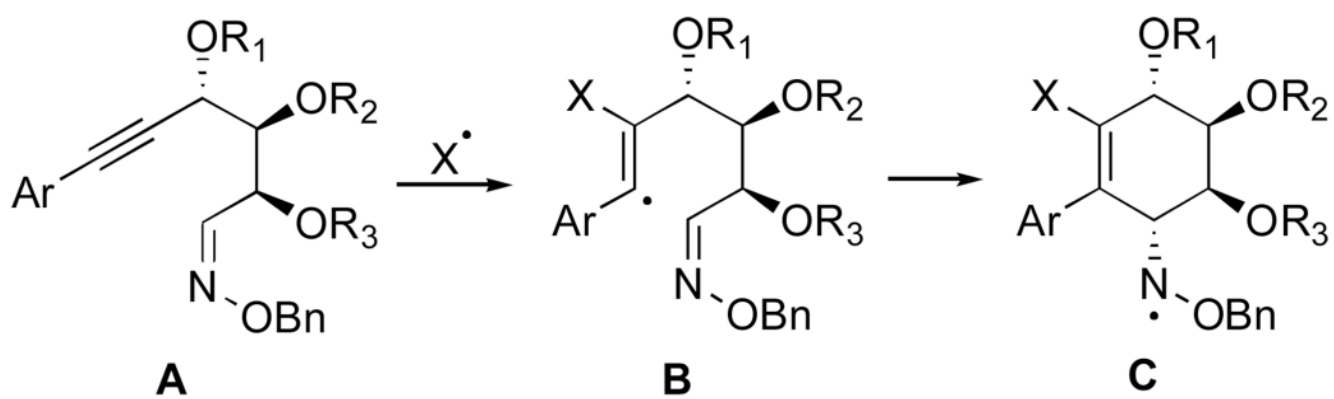
Figure 23.
Radical cyclization strategy to (+)-narciclasine and (+)-7-deoxynarciclasine.
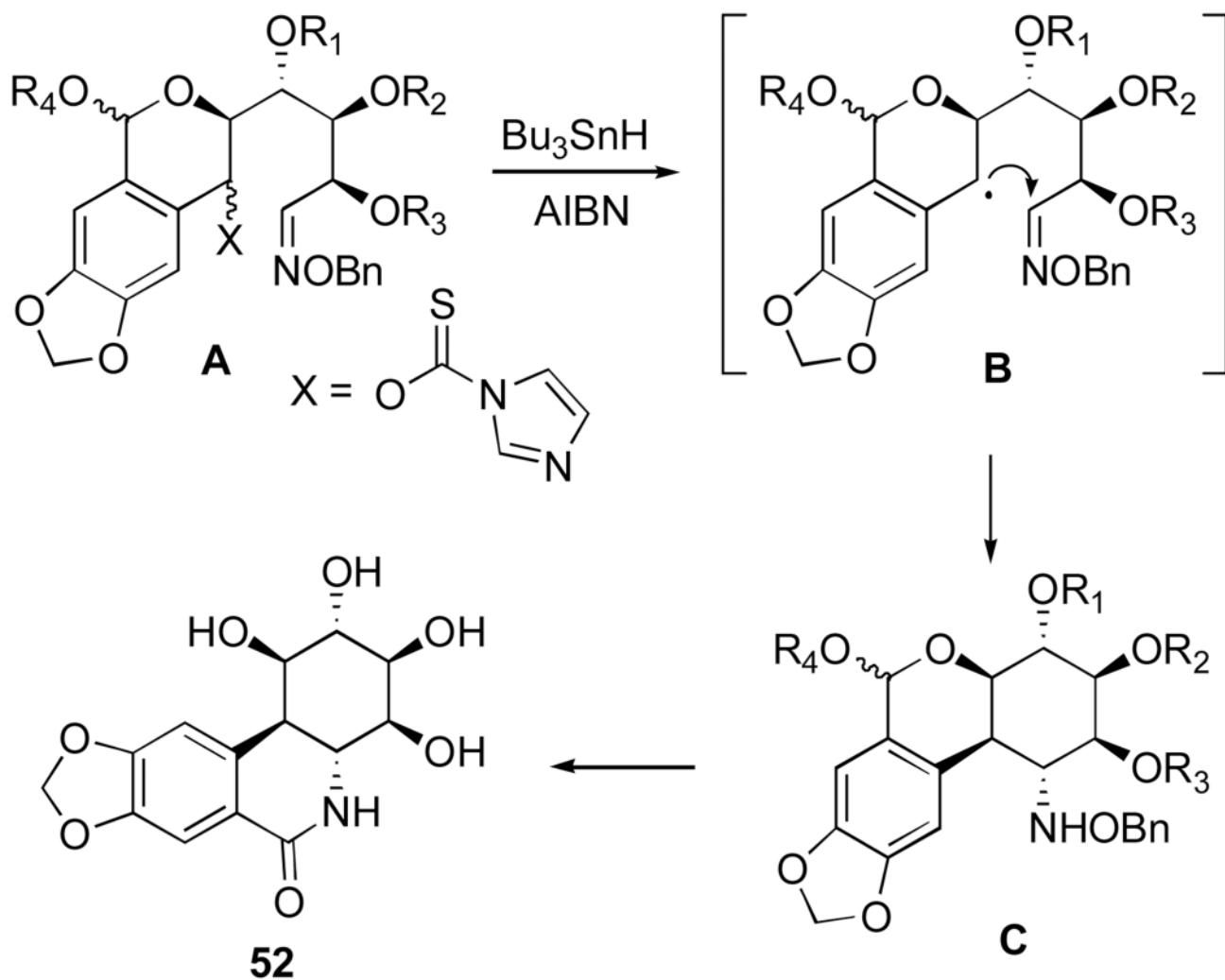
Figure 24.
Radical cyclization strategy in the first generation synthesis of (+)-7-deoxypancratistatin of Keck and coworkers.
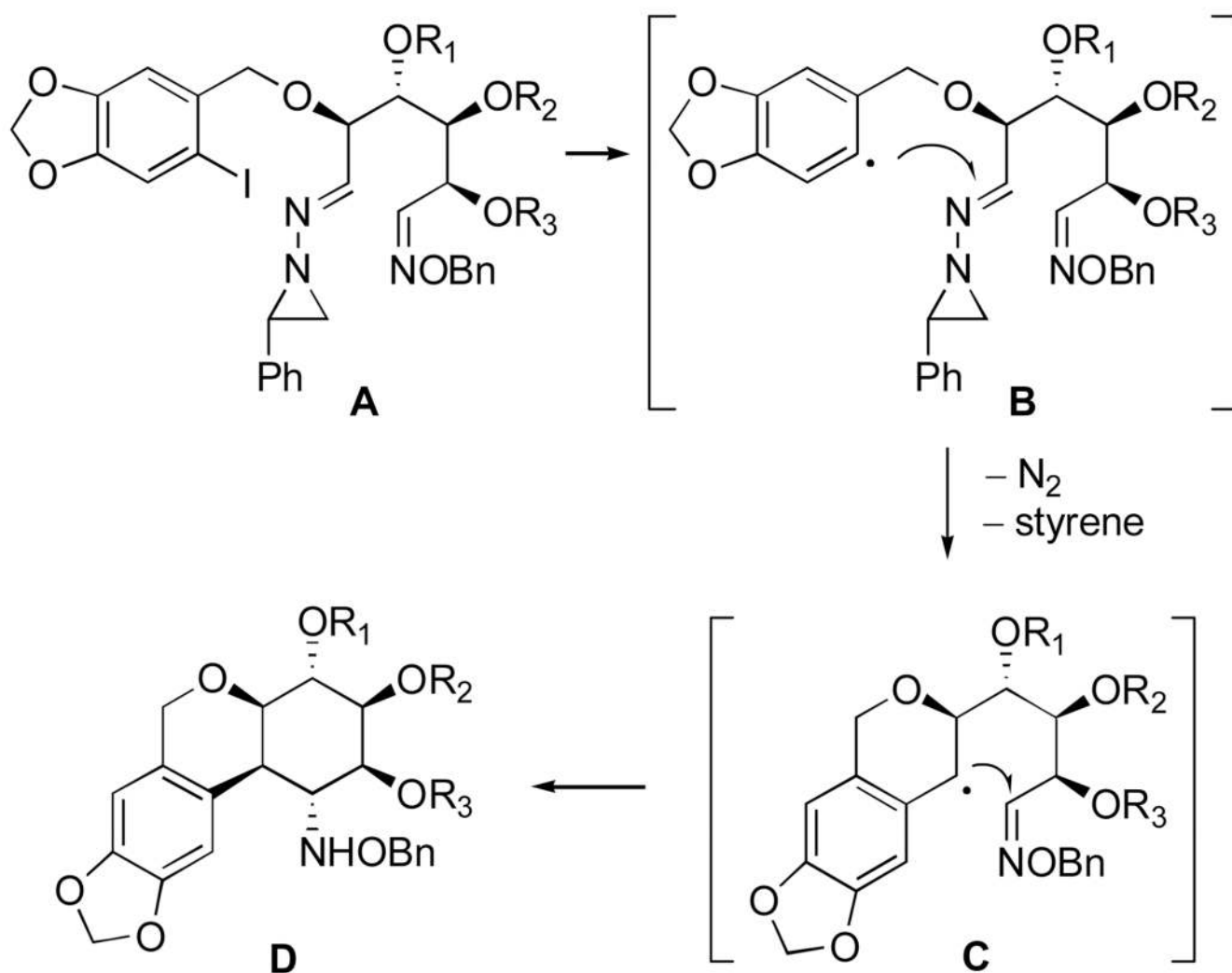
Figure 25.
Kim cascade radical cyclization strategy in the second generation synthesis of (+)-7-deoxypancratistatin of Keck and coworkers.
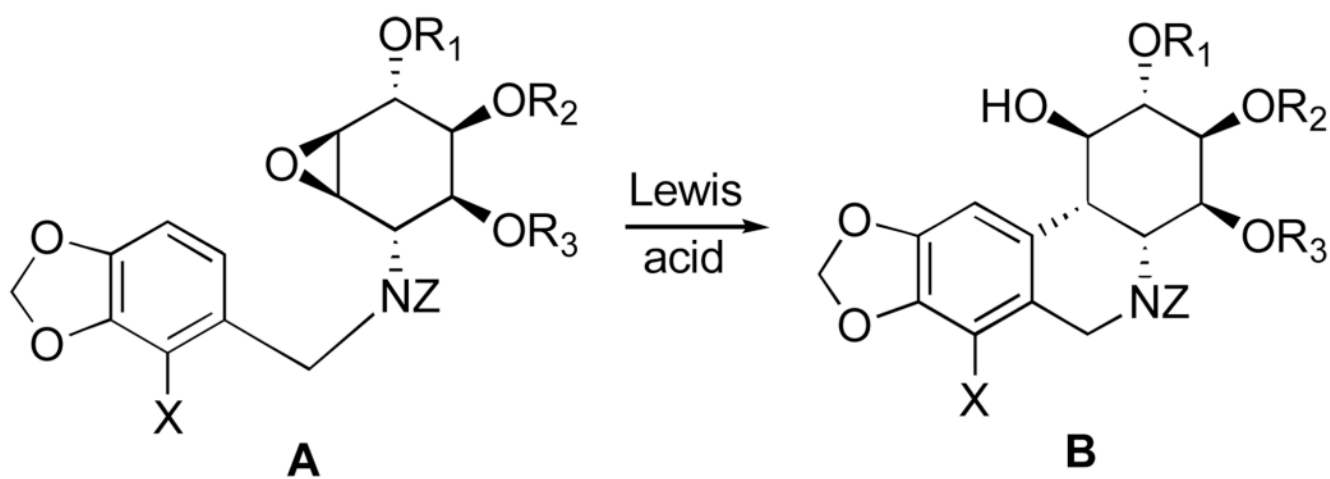
Figure 26.
Strategy to (+)-narciclasine based on intramolecular arene-epoxide ring opening.
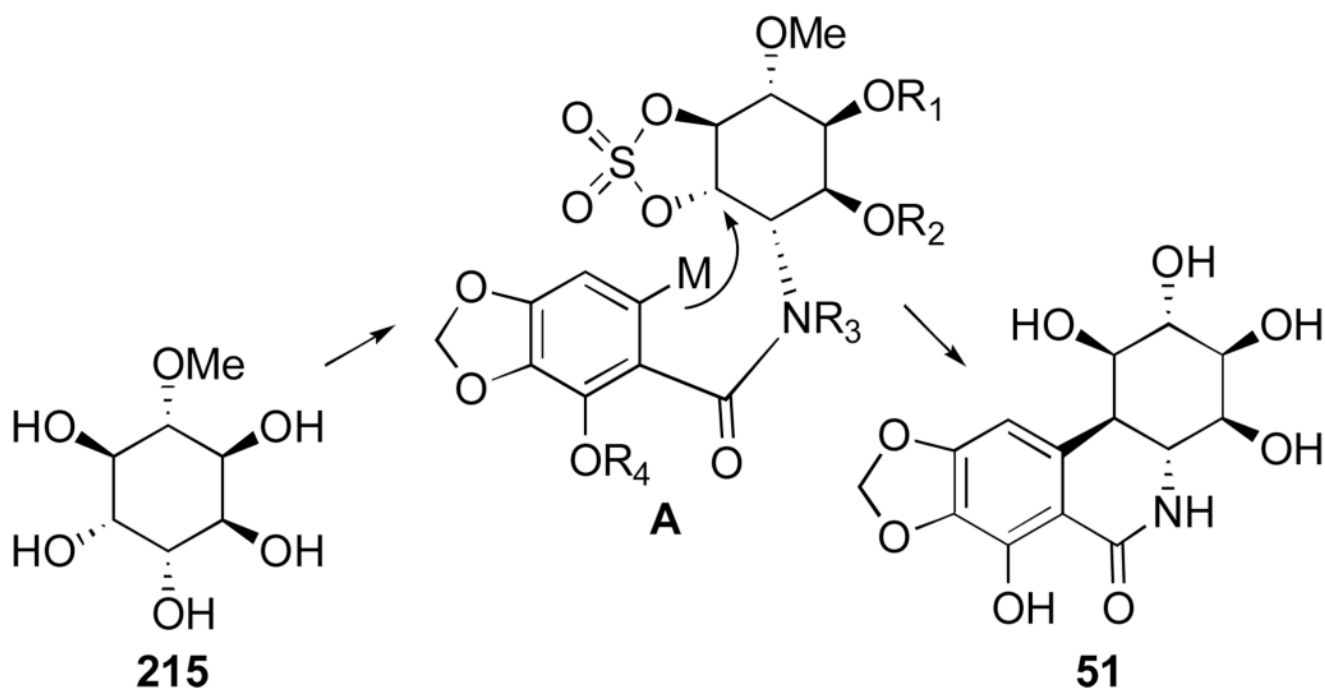
Figure 27.
Strategy to (+)-pancratistatin based on intramolecular ring opening of a cyclic sulfate
moiety.
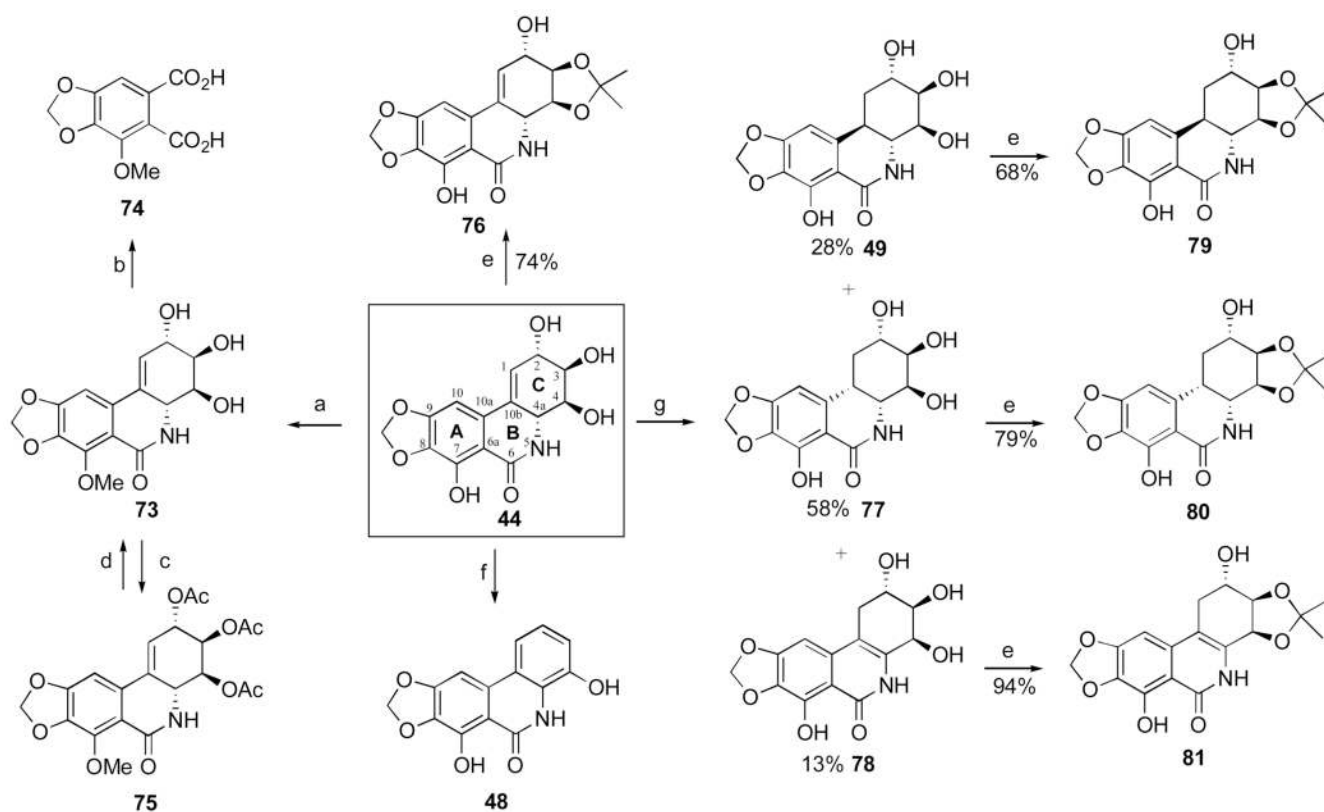
Scheme 1.a.
[a] Reagents and conditions: (a) CH_2N_2 (excess), EtOH, 48 h; (b) $KMnO_4$, H_2O, 0 °C, 48 h; (c) Ac_2O, py, 48 h, rt; (d) $Ba(OH)_2$, MeOH, reflux; (e) acetone, $HC(OEt)_3$, TsOH, reflux, 15 h; (f) conc. HCl; (g) H_2, $Pd/CaCO_3$, EtOH, 3 h.
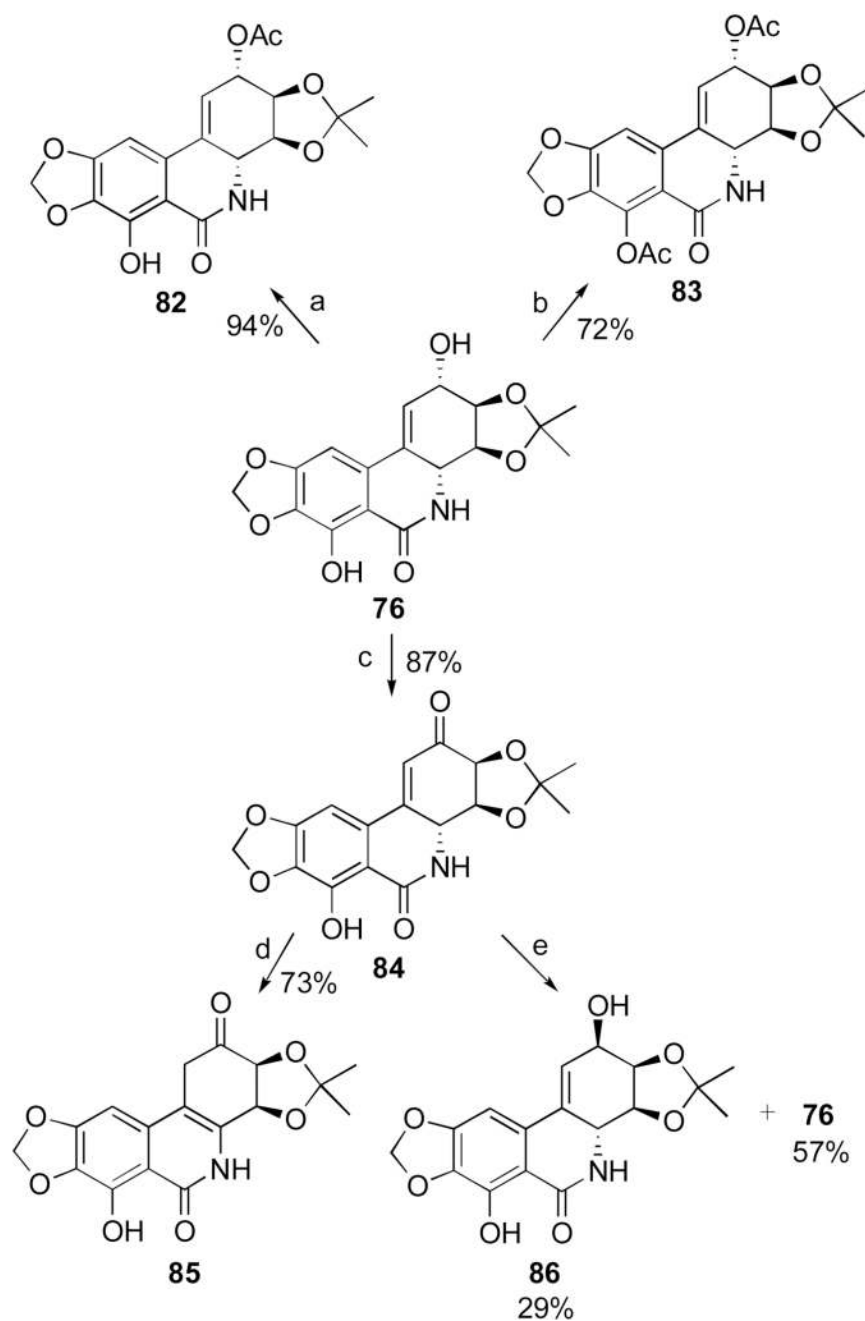
Scheme 2.a.
^a Reagents and conditions: (a) Ac$_2$O, py, 3 h; (b) Ac$_2$O, py, 3 d; (c) MnO$_2$, THF, 2.5 h; (d) silica gel, THF; (e) NaBH$_4$, EtOH, 0.5 h, rt.
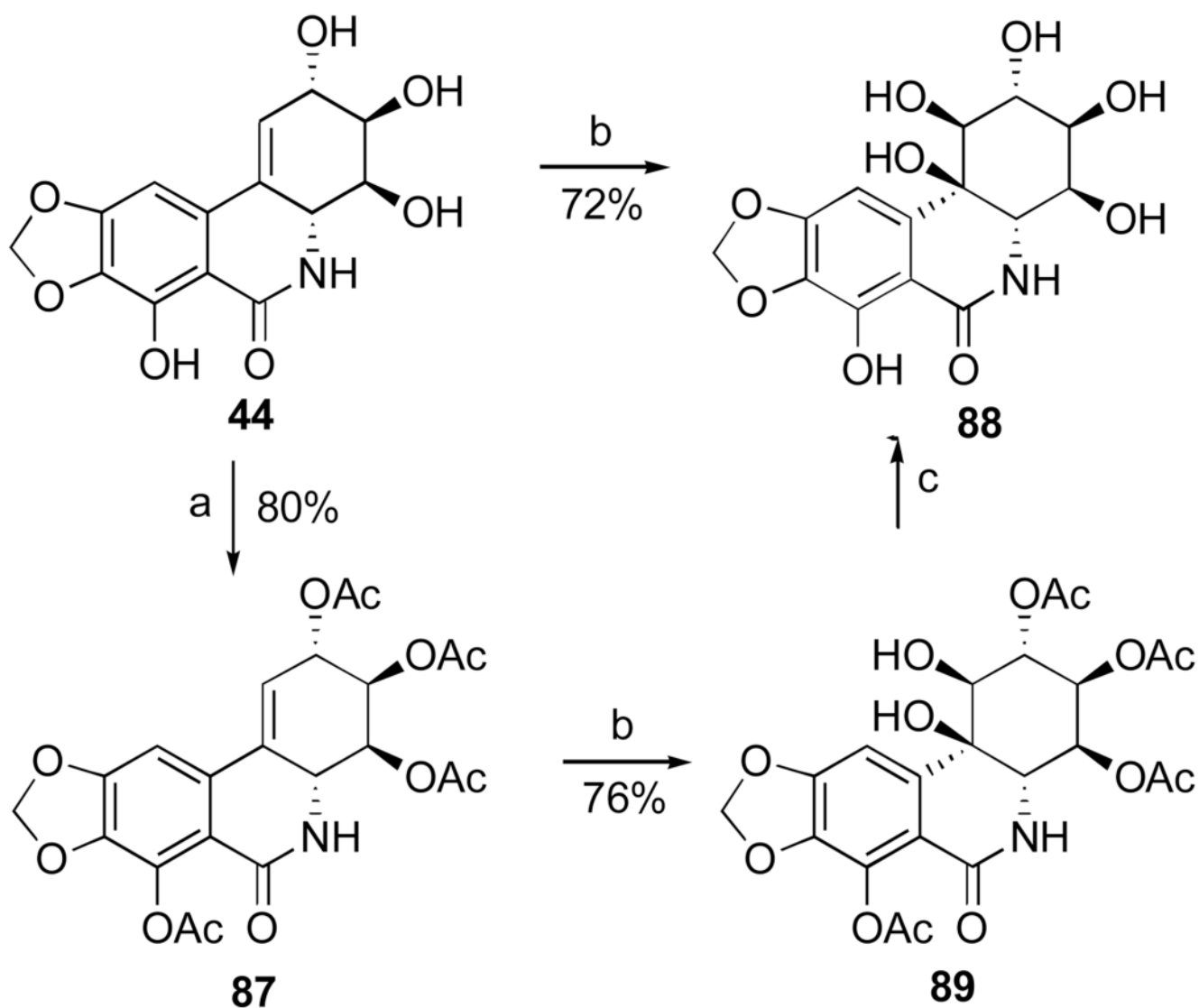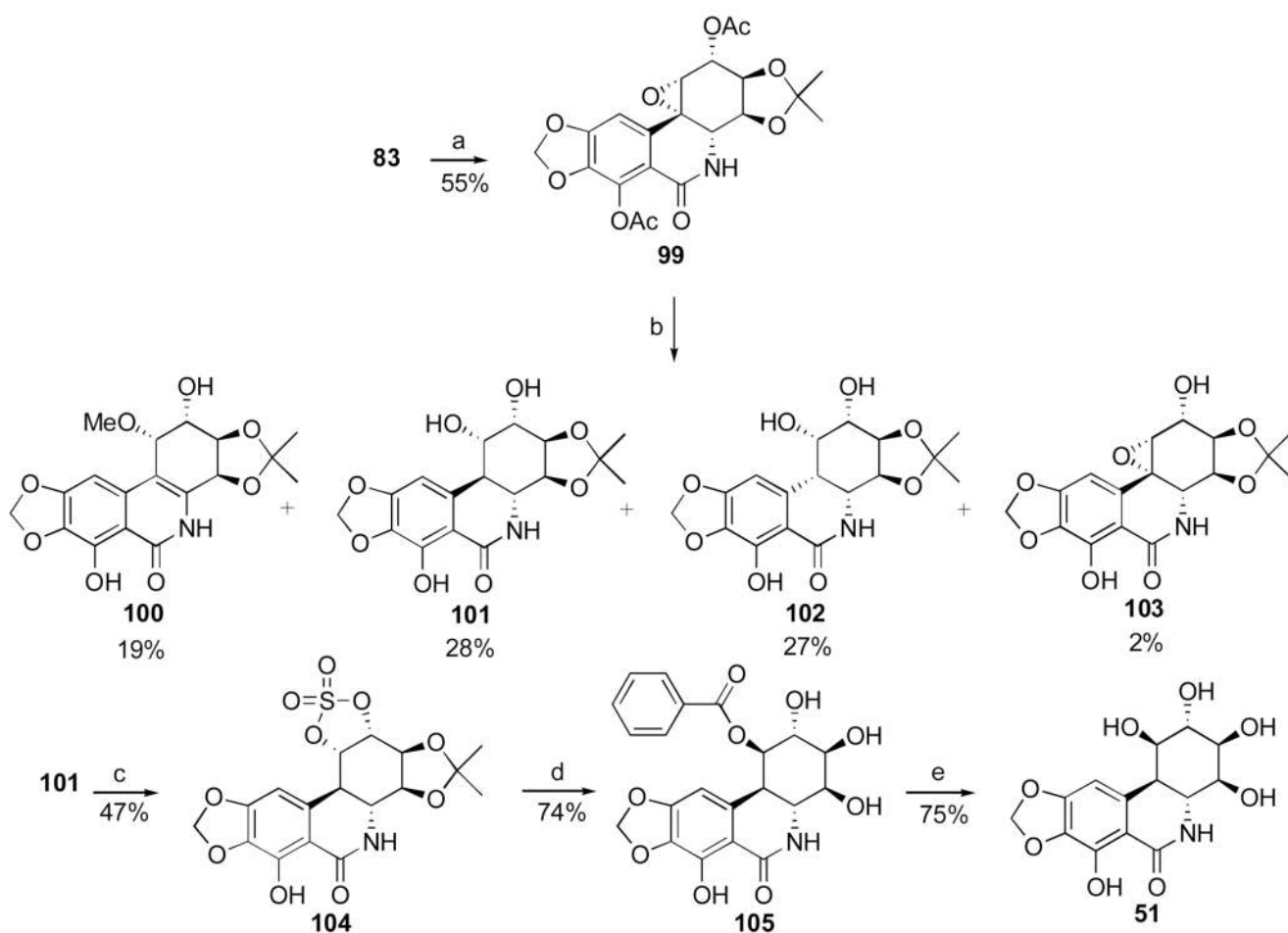
Scheme 3.a.
[a] Reagents and conditions: (a) Ac_2O, py; (b) OsO_4 (cat.), NMO, HQ, DMF/H_2O; (c) $NH_3/$ MeOH, CH_2Cl_2.
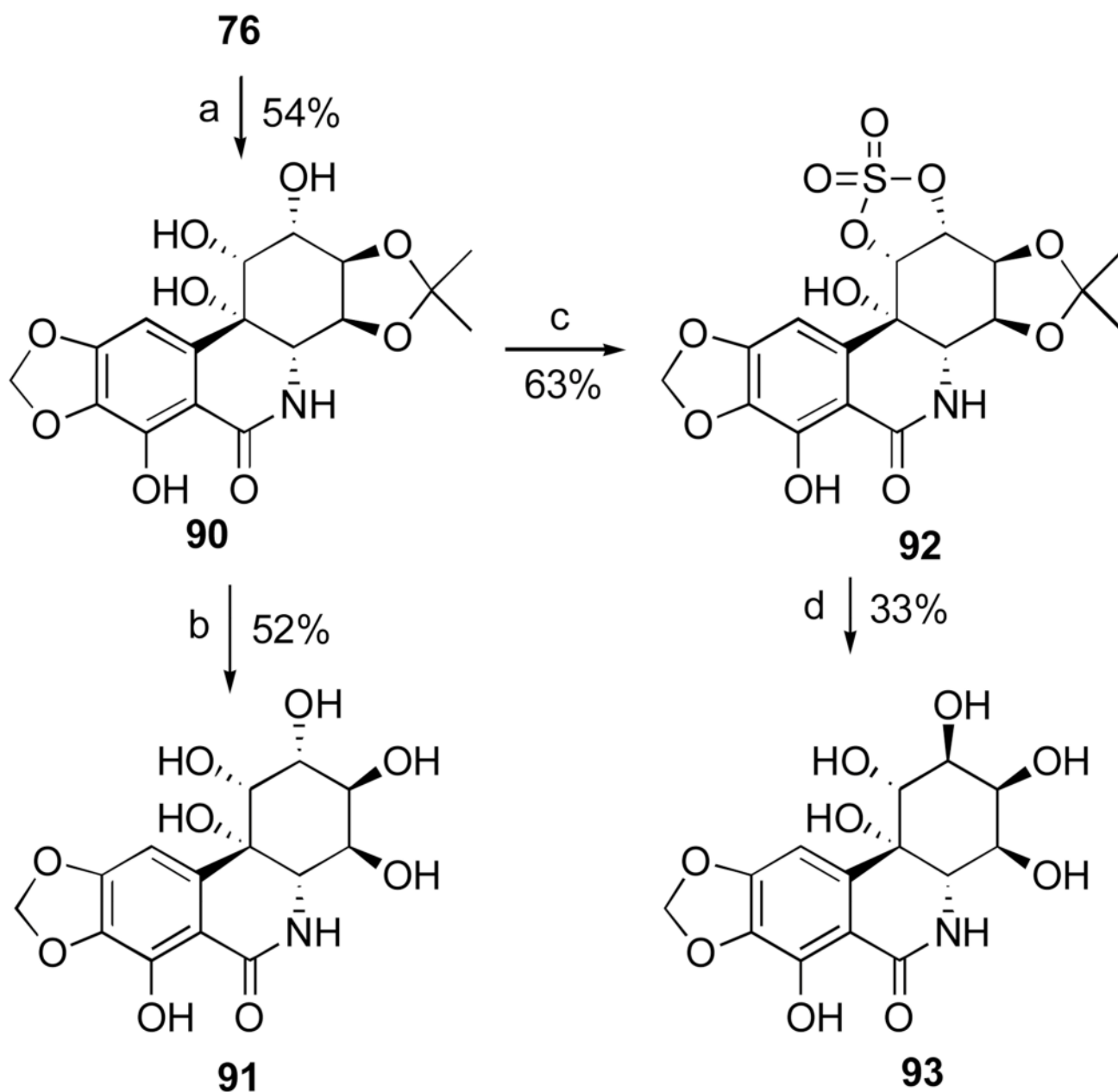
Scheme 4.a.
[a] Reagents and conditions: (a) OsO_4 (cat.), NMO, $(DHQD)_2PHAL$, DMF/H_2O; (b) H_2SO_4, THF/H_2O; (c) $SOCl_2$, Et_3N, THF; $RuCl_3 \cdot 3H_2O/NaIO_4$, $MeCN/CCl_4/H_2O$; (d) $PhCO_2H$, Cs_2CO_3, DMF; H_2SO_4, THF/H_2O, 60 °C; K_2CO_3, MeOH.
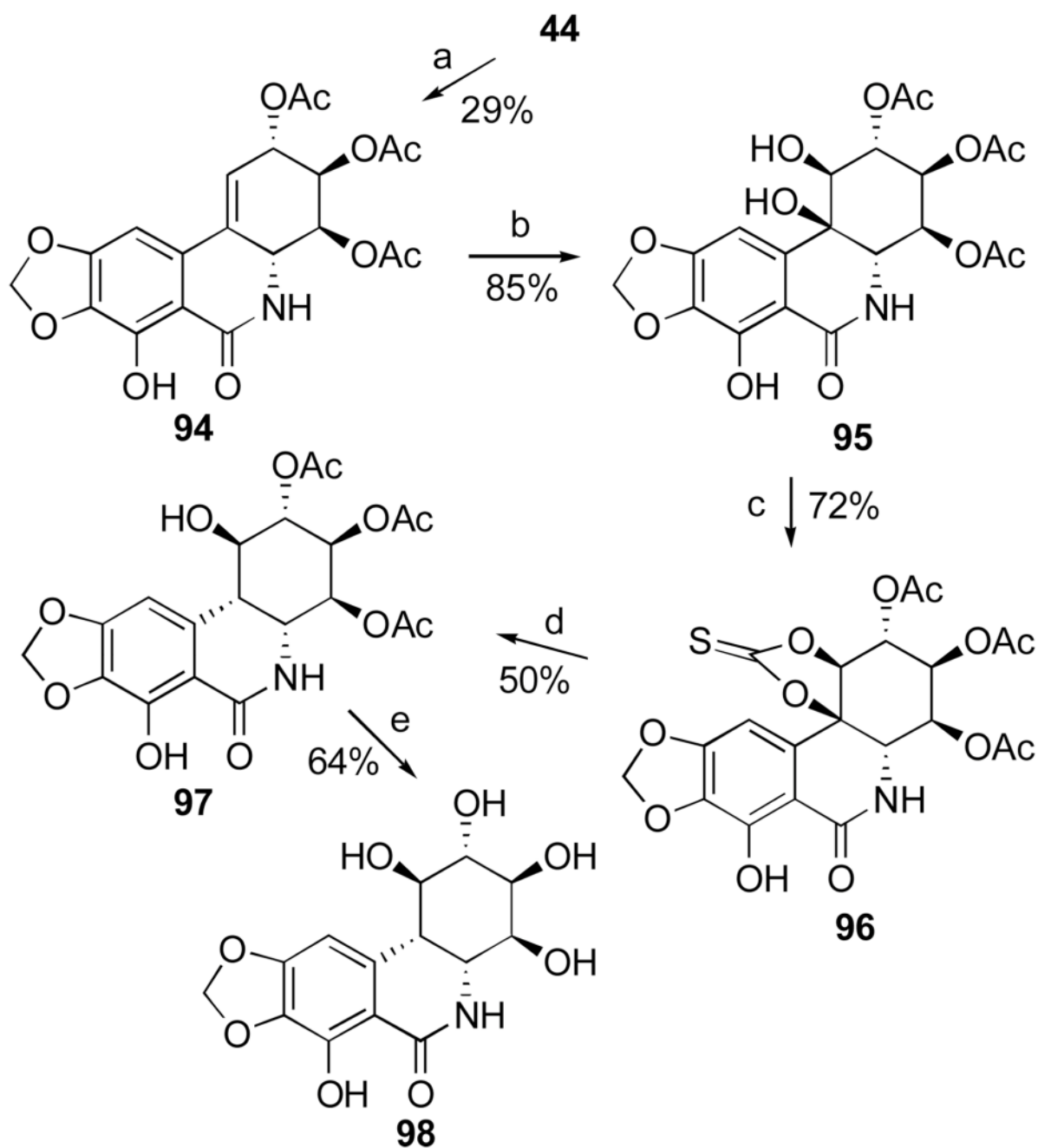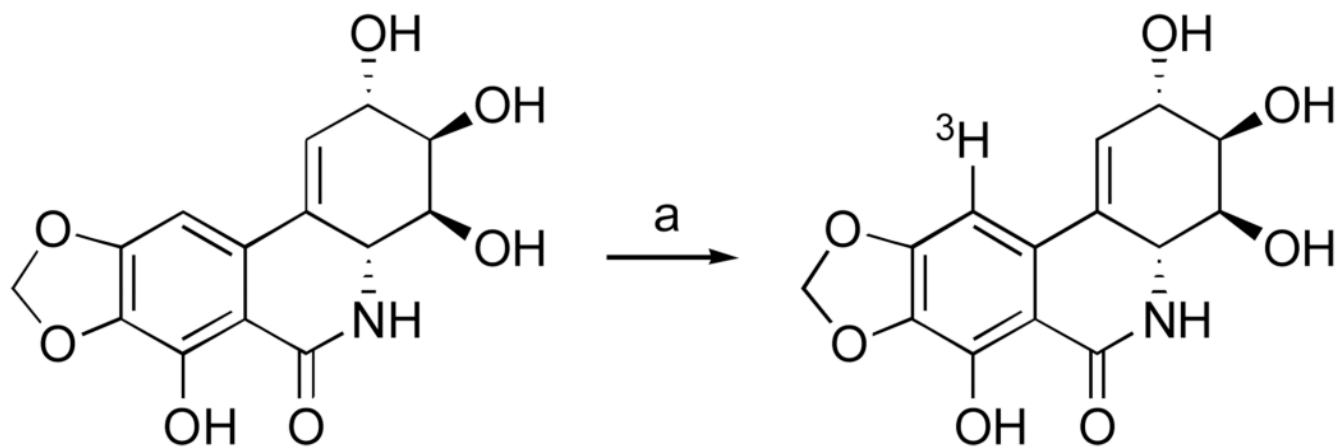
Scheme 5.a.
^{a} Reagents and conditions: (a) Ac_2O (3.25 equiv.), py; (b) OsO_4 (cat.), NMO, (DHQ)$_2$PHAL, DMF/H_2O; (c) *N,N'*-thiocarbonyldiimidazole, 2-butanone; (d) Bu$_3$SnH, AIBN, PhCH$_3$, 80 °C, 16 h; (e) K_2CO_3, H_2O/MeOH/CH_2Cl_2.

Scheme 6.a.

[a] Reagents and conditions: (a) MCPBA, phosphate buffer; (b) H_2, 10% Pd/C; K_2CO_3, MeOH/H_2O (c) $SOCl_2$, Et_3N, THF; $RuCl_3 \cdot 3H_2O$/$NaIO_4$, MeCN/CCl_4/H_2O; (d) $PhCO_2H$, Cs_2CO_3, DMF; H_2SO_4, THF/H_2O, 70 °C; (e) K_2CO_3, MeOH.
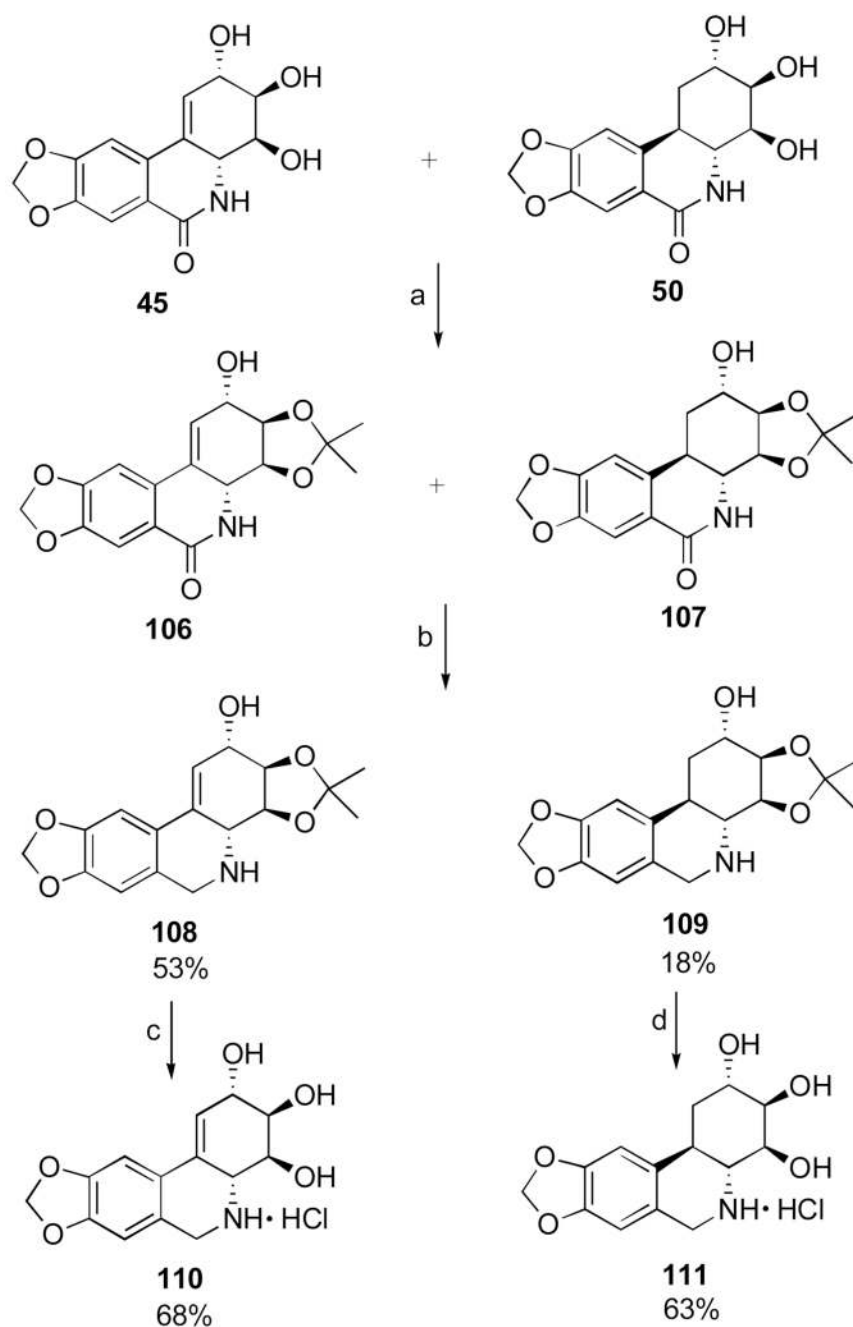
Scheme 7.a.

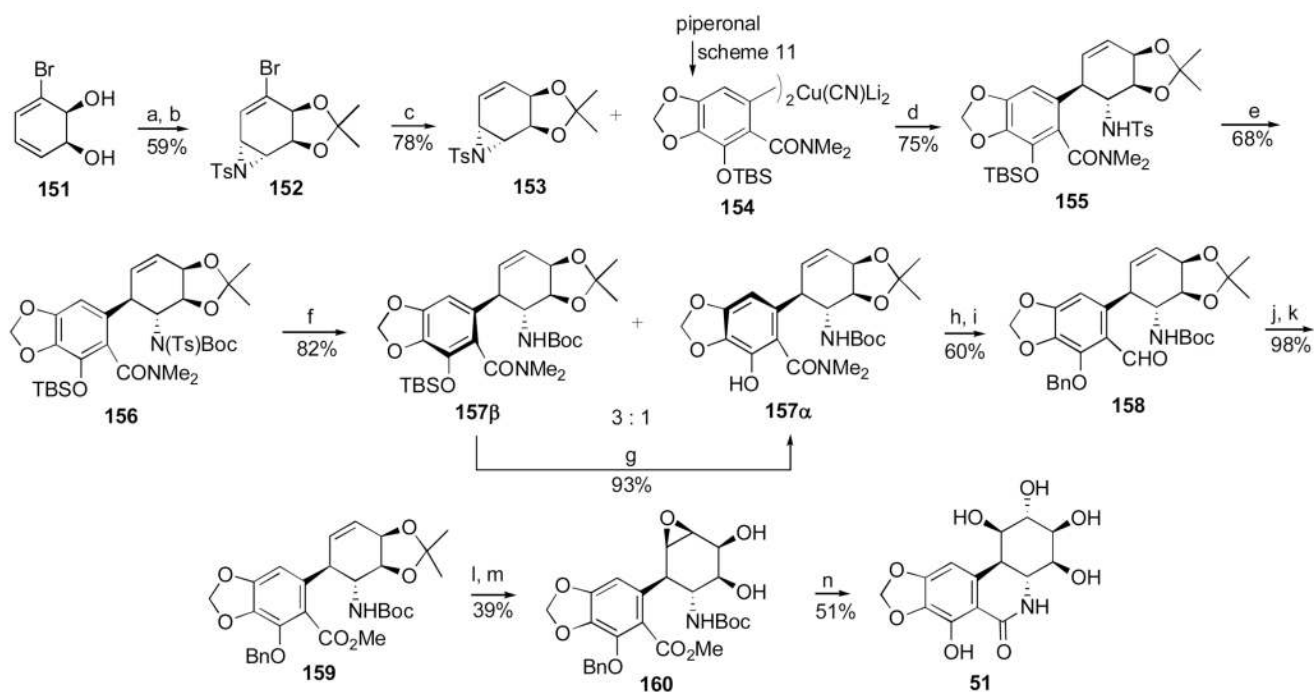[a] Reagents and conditions: (a) $Me_2CH(OMe)_2$, *p*-TsOH, DMF; (b) TBSCl, imidazole, DMF; $LiAlH_4$, ether; TBAF, THF; (c) aq. H_2SO_4, THF/CH_2Cl_2; $NaHCO_3$; HCl, MeOH (d) HCl, MeOH.
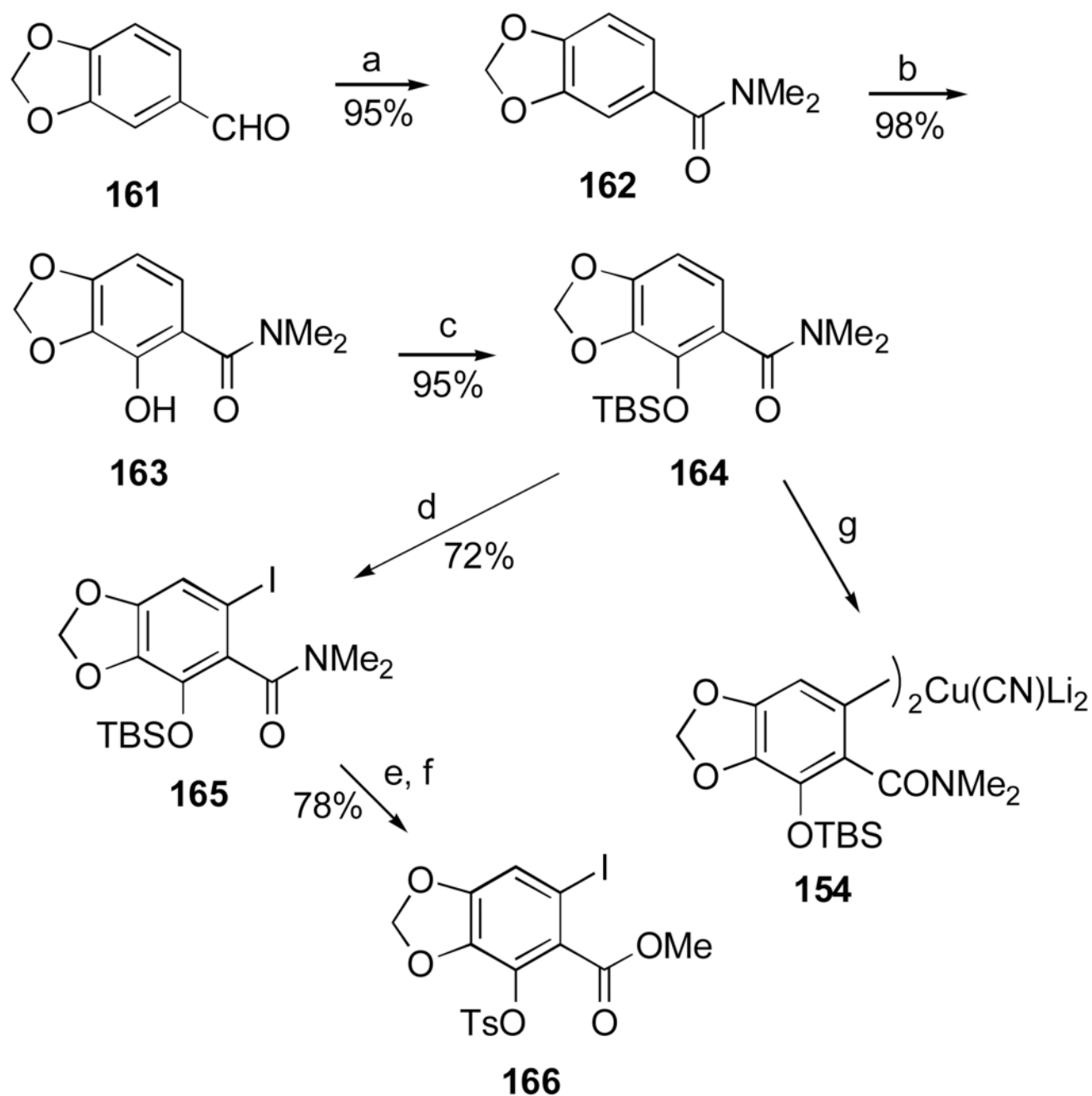
Scheme 8.a.
a Reagents and conditions: (a) ^3H_2O, Pd/C, 60 °C, 23 h, sealed ampoule; co-evaporation with ethanol (5 times).
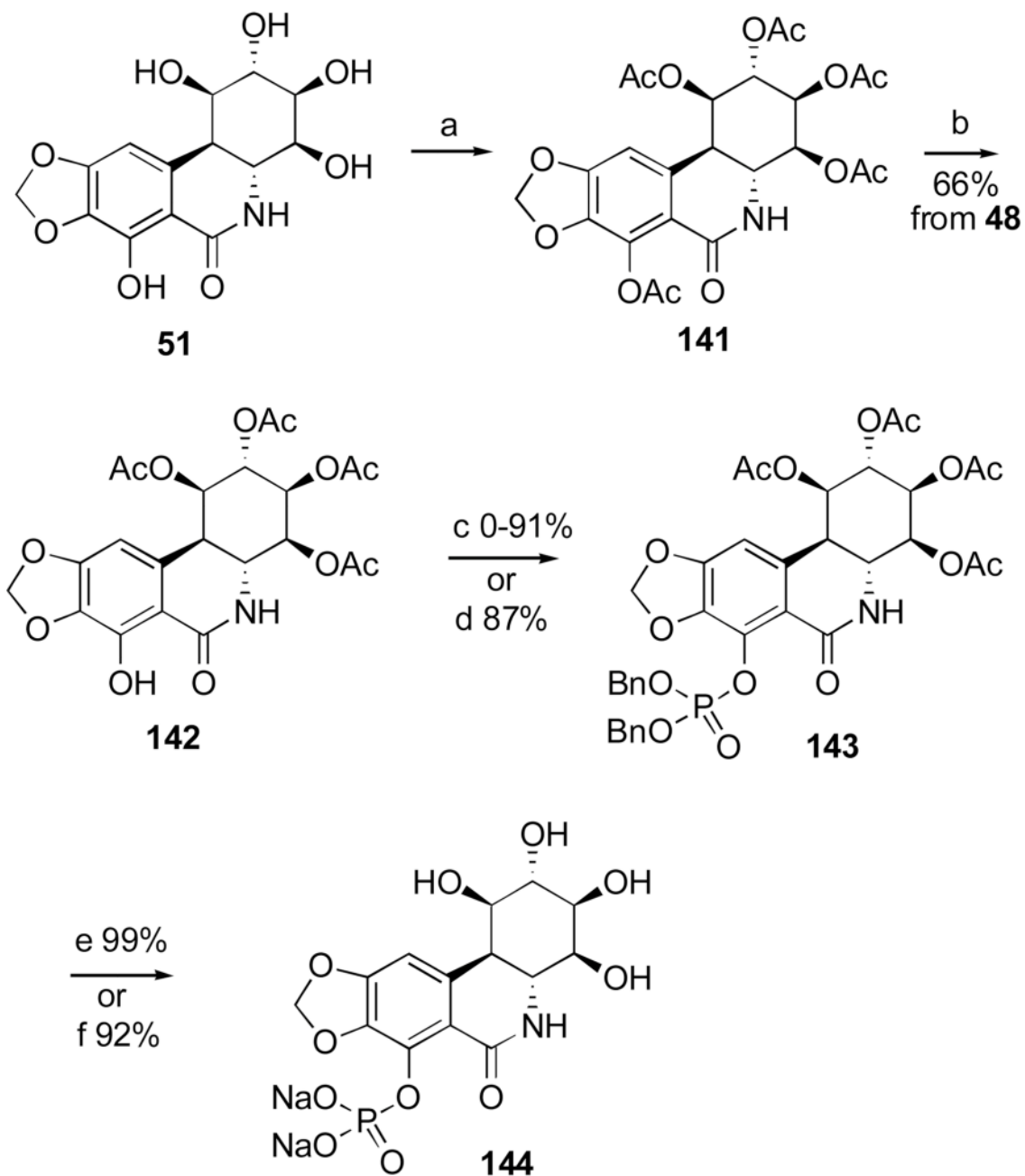
Scheme 9.a.
[a] Reagents and conditions: (a) Ac$_2$O, DMAP, py; (b) H$_2$O, py, reflux; (c) i-Pr$_2$NP(OBn)$_2$, tetrazole, CH$_2$Cl$_2$; MCPBA, CH$_2$Cl$_2$; (d) (BnO)$_2$P(O)H, DIPEA, DMAP, CCl$_4$/MeCN; (e) H$_2$, 10% Pd/C, ethanol; NaOH, H$_2$O; (f) NaOMe, HOMe; H$_2$, 10% Pd/C, ethanol; NaOMe, HOMe.

Scheme 10.a.
a Reagents and conditions: (a) $Me_2C(OMe)_2$, p-TsOH, CH_2Cl_2; (b) PhI=NTs, $Cu(acac)_2$, MeCN; (c) Bu_3SnH, AIBN, THF; (d) THF, −78 °C to rt; (e) s-BuLi, THF, $(Boc)_2O$; (f) Na/ anthracene, DME, −78 °C; (g) TBAF, THF; (h) $NaAl(MeOCH_2CH_2O)_2H_2$, 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, $VO(acac)_2$, PhH, 60 °C; (n) BzONa (cat.), H_2O, 100 °C.

Scheme 11.a.
a Reagents and conditions: (a) MnO$_2$, NaCN, Me$_2$NH, i-PrOH; (b) n-BuLi, TMEDA, −104 °C; B(OMe)$_3$, THF; AcOH, H$_2$O$_2$; (c) TBSCl, imidazole; (d) n-BuLi, TMEDA, −104 °C, Et$_2$O, I$_2$; (e) Me$_3$OBF$_4$, Na$_2$HPO$_4$, MeCN; (f) TsCl, py; (g) i. s-BuLi, TMEDA, THF, −90 °C; ii. CuCN, −90 to −20 °C.
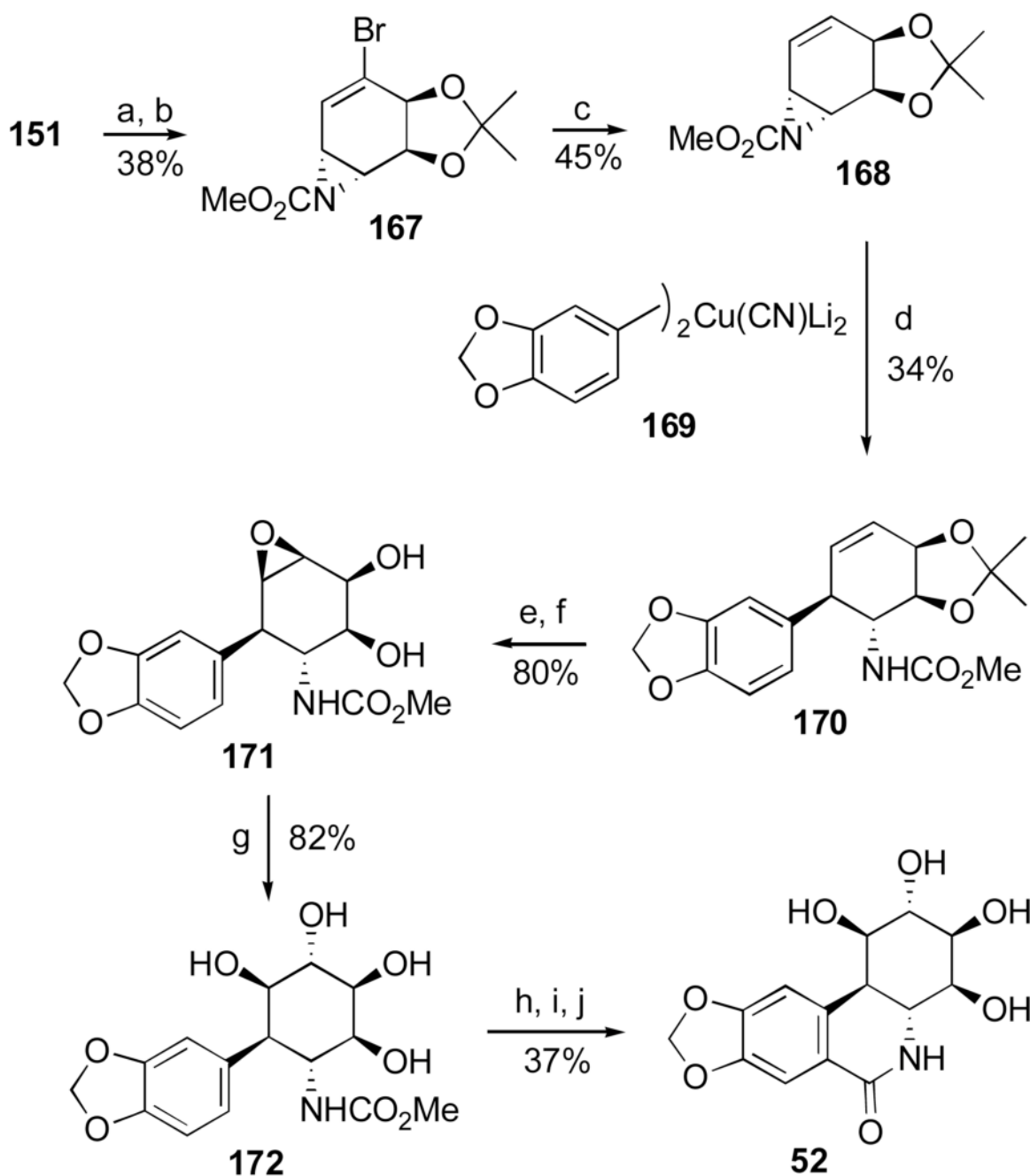
Scheme 12.a.
^a Reagents and conditions: (a) Me₂C(OMe)₂, *p*-TsOH, CH₂Cl₂; (b) *p*-O₂NPhSO₃NHCO₂Me, K₂CO₃, CH₂Cl₂; (c) Bu₃SnH, AIBN, THF; (d) BF₃·Et₂O, THF, −78 °C to −30 °C; (e) AcOH, THF, H₂O, 65 °C; (f) *t*-BuOOH, VO(acac)₂, PhH, 60 °C; (g) BzONa (cat.), H₂O, 100 °C; (h) Ac₂O, DMAP, py; (i) Tf₂O, DMAP, CH₂Cl₂, 5 °C; (j) MeONa, MeOH.
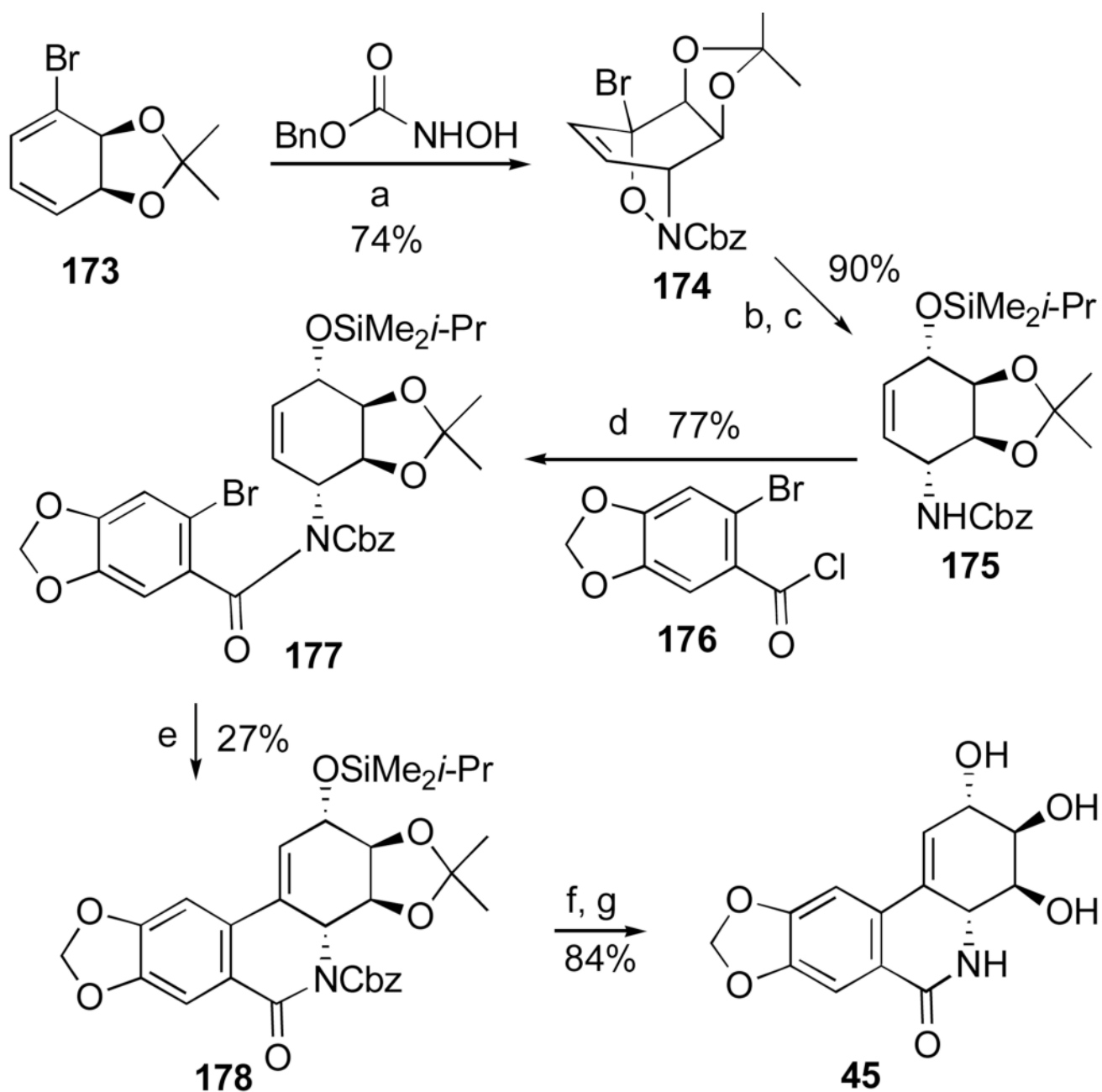
Scheme 13.a.

a Reagents and conditions: (a) Bu_4NIO_4, CH_2Cl_2; (b) Al(Hg), THF; (c) i-$PrMe_2SiCl$, imidazole, CH_2Cl_2; (d) BuLi, THF, −78 °C; (e) $Pd(OAc)_2$, Tl(OAc), DIPHOS, anisole; (f) Pd/C, cyclohexene, EtOH; (g) CF_3CO_2H, 0 °C.
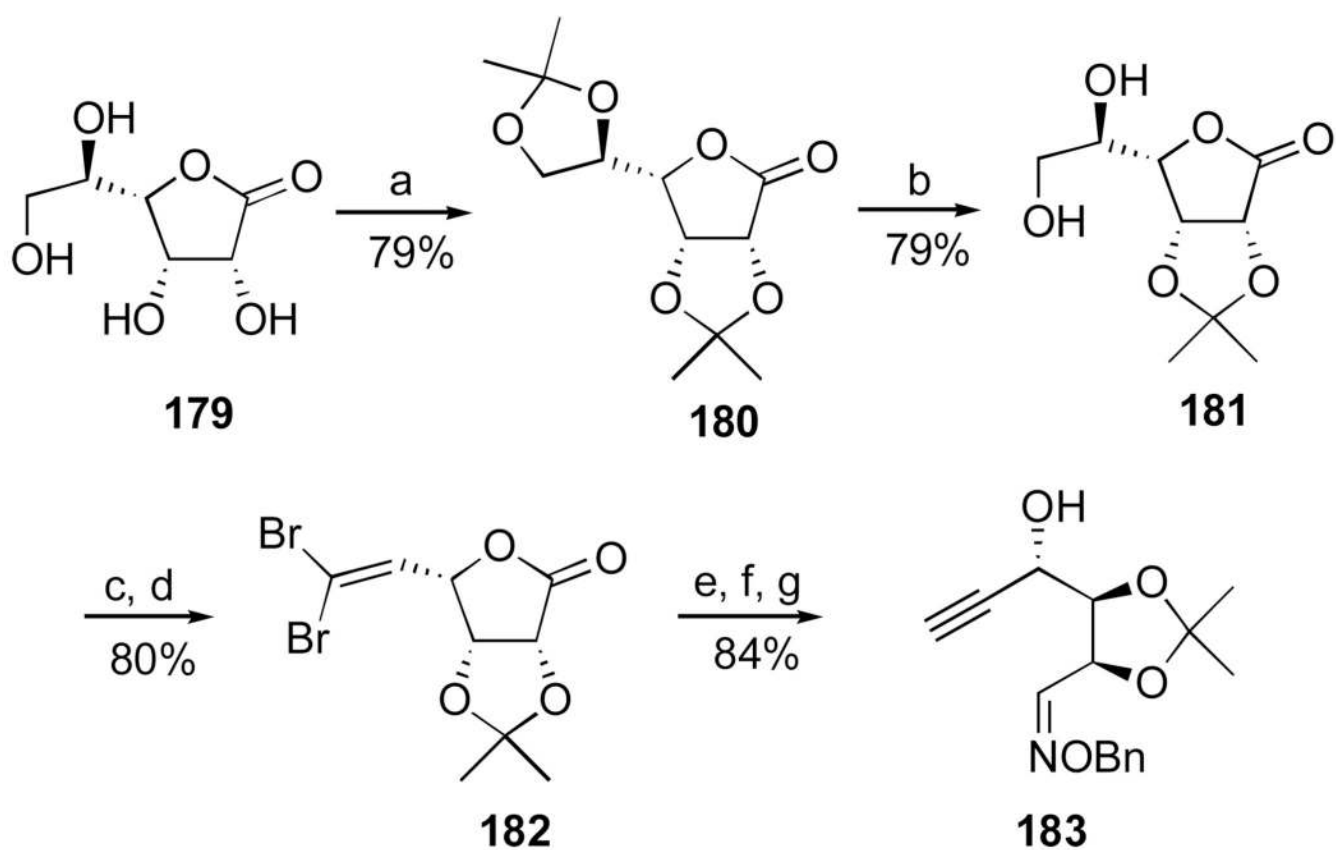
Scheme 14.a.
[a] Reagents and conditions: (a) $Me_2C(OMe)_2$, p-TsOH; (b) AcOH, H_2O; (c) $NaIO_4$, CH_2Cl_2; (d) CBr_4, PPh_3, NEt_3; (e) L-selectride, Et_2O, −78 °C; (f) HCl·H_2NOBn, py; (g) n-BuLi, Et_2O, −90 °C.
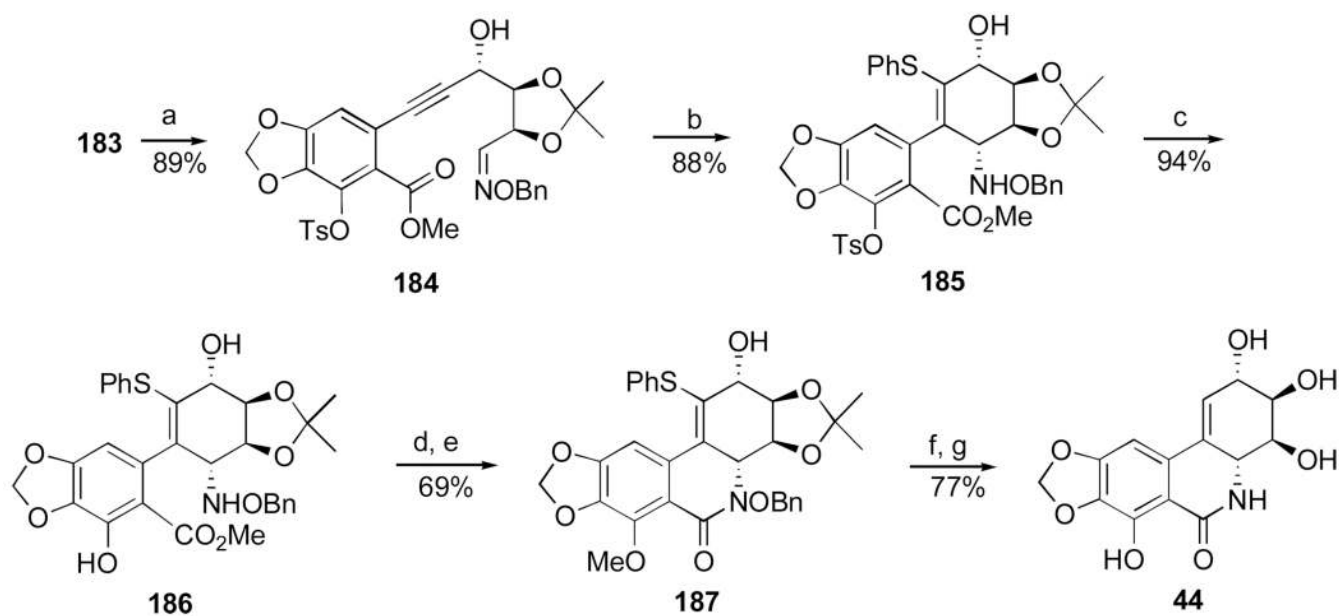
Scheme 15.a.

a Reagents and conditions: (a) **166**, Pd(OAc)$_2$, CuI, NEt$_3$, PPh$_3$, THF; (b) PhSH, hv, PhMe, 27 °C; (c) SmI$_2$, THF, H$_2$O, 0 °C; (d) MeI, K$_2$CO$_3$, DMF; (e) Me$_3$Al, THF; (f) SmI$_2$, MeOH, THF, 0 °C; (g) TFA, 0 °C.
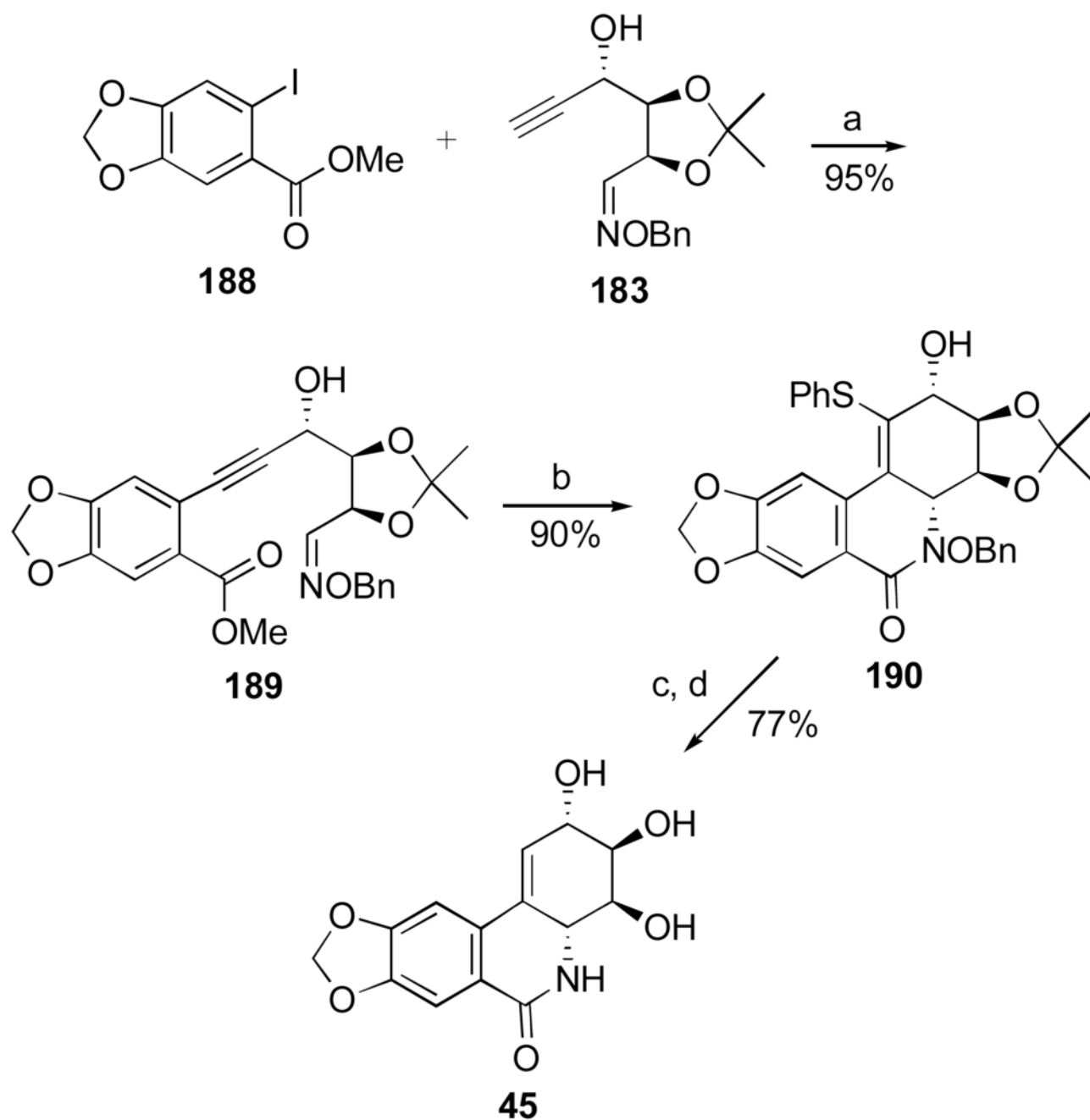
Scheme 16.a.
[a] Reagents and conditions: (a) Pd(OAc)$_2$, CuI, NEt$_3$, PPh$_3$, THF; (b) PhSH, hv, PhMe, 27 °C; (c) SmI$_2$, THF; (d) TFA, 0 °C.
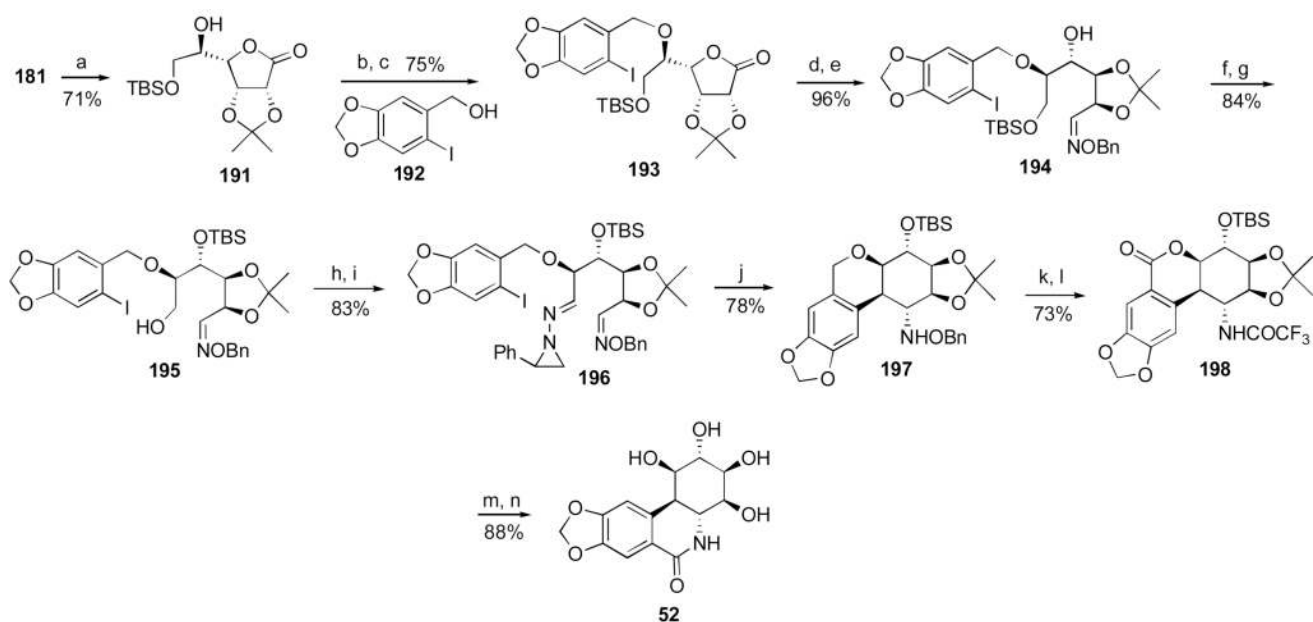
Scheme 17.a.
a Reagents and conditions: (a) TBSCl, imidazole; (b) **192**, NaH, Cl₃CCN, 0 °C; (c) TfOH, THF, 0 °C; (d) L-selectride, CH₂Cl₂, −78 °C; (e) HCl·H₂NOBn, py; (f) TBSOTf, 2,6-lutidine, CH₂Cl₂, 0 °C; (g) HF·py, THF; (h) TPAP, NMO, 4Å MS; (i) 1-amino-2-phenylaziridine, EtOH, 0 °C; (j) Ph₃SnH, AIBN, PhH, 78 °C; (k) SmI₂, THF, then TFAA; (l) PCC, CH₂Cl₂, 55 °C; (m) BF₃·Et₂O, CH₂Cl₂; (n) K₂CO₃, MeOH.
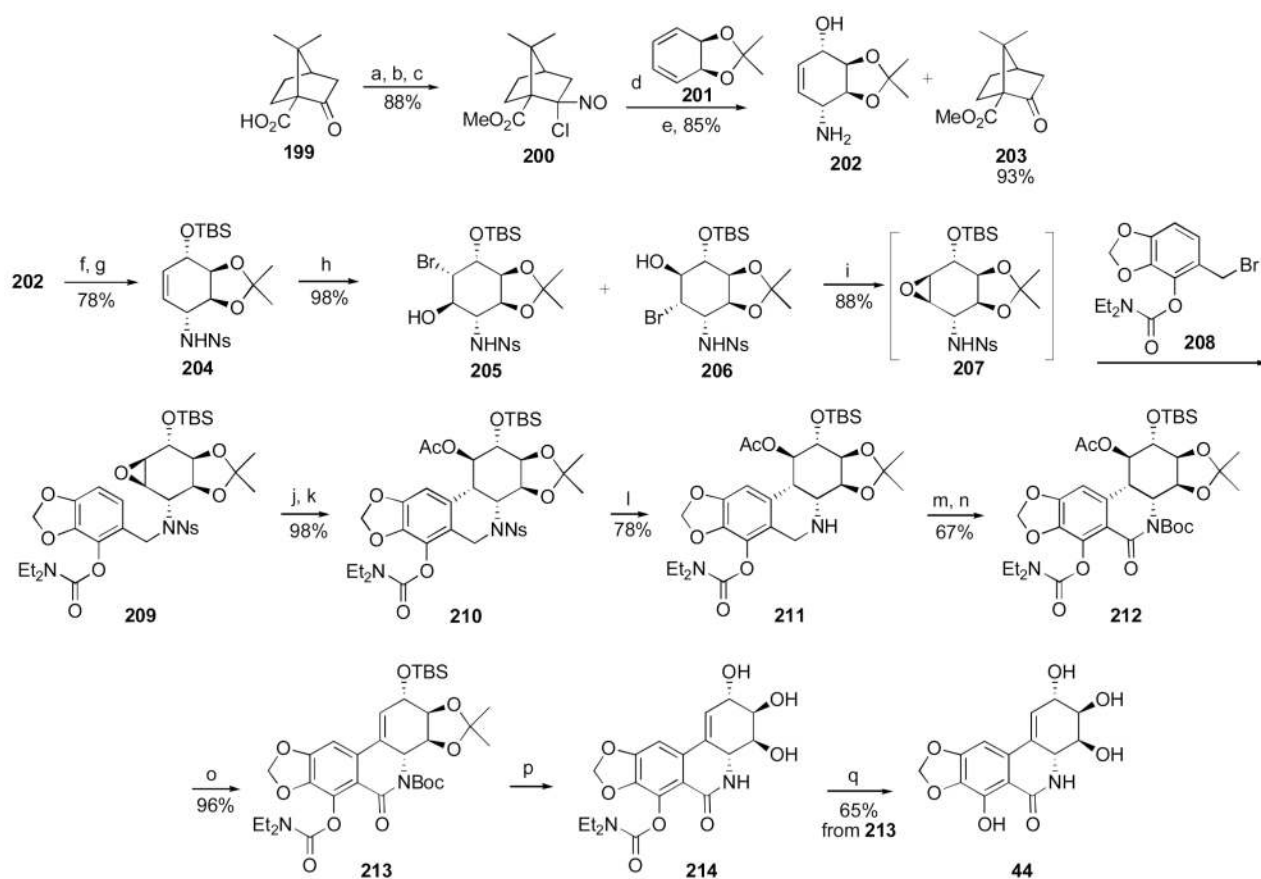
Scheme 18.a.
a Reagents and conditions: (a) SOCl$_2$, MeOH; (b) HCl·NH$_2$OH, MeOH, AcONa; (c) *t*-BuOCl, CH$_2$Cl$_2$; (d) CH$_2$Cl$_2$, 0 °C, 12 h; (e) Al-Hg, H$_2$O/MeCN; (f) *p*-O$_2$NPhSO$_2$Cl, MeCN, NEt$_3$; (g) TBSCl, DBU; (h) NBS, H$_2$O/acetone; (i) MeCN, K$_2$CO$_3$, 60 °C, then **208**; (j) 20 mol% SnCl$_4$, CH$_2$Cl$_2$; (k) Ac$_2$O, K$_2$CO$_3$; (l) HSCH$_2$CO$_2$H, LiOH, DMF; (m) Boc$_2$O, MeCN; (n) RuCl$_3$, NaIO$_4$, H$_2$O; (o) DBU, PhH, 70 °C; (p) HCO$_2$H, THF, 60 °C; (q) LiAlH$_4$, THF.
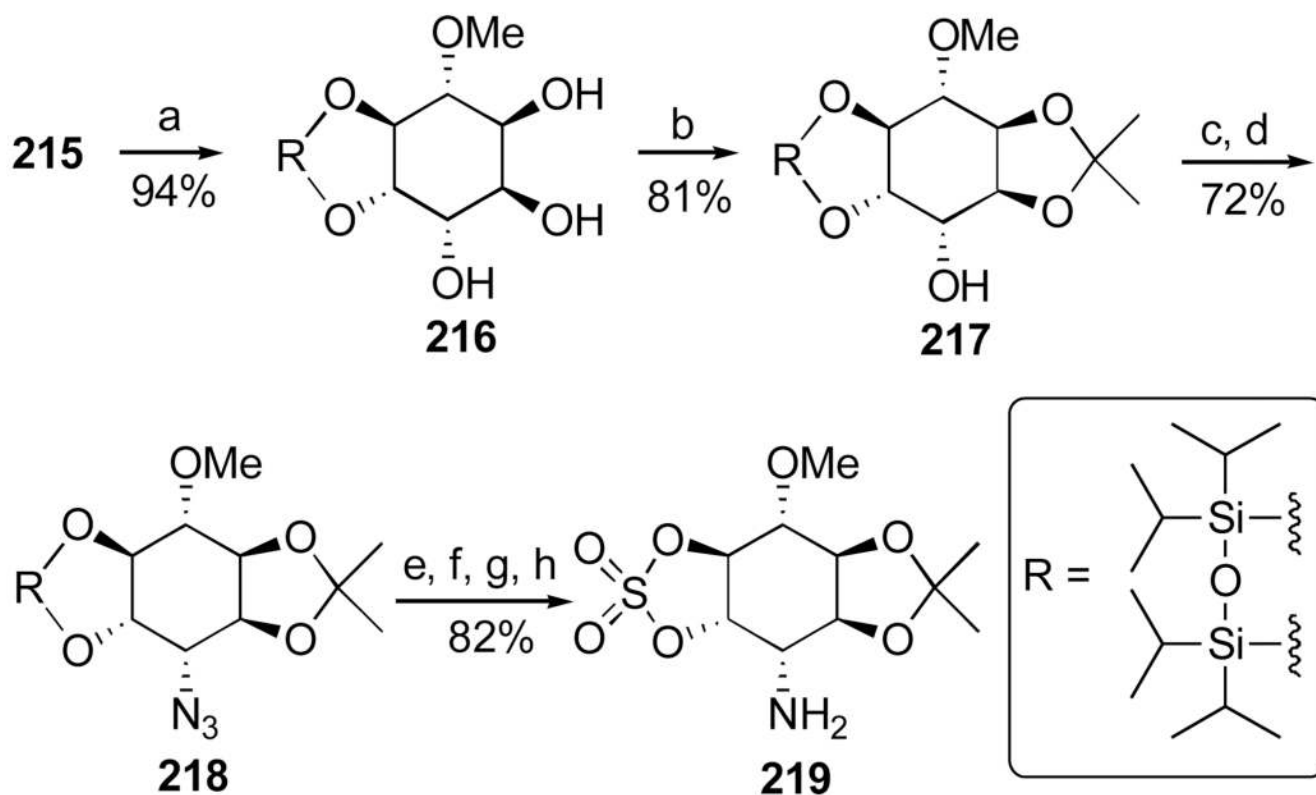
Scheme 19.a.
a Reagents and conditions: (a) TIPDSCl$_2$, imidazole, DMAP; (b) Me$_2$C(OMe)$_2$, *p*-TsOH; (c) PPh$_3$, DEAD, MeSO$_3$H, CH$_2$Cl$_2$; (d) NaN$_3$, DMF, 60 °C; (e) TBAF, THF; (f) SOCl$_2$, Et$_3$N, CH$_2$Cl$_2$; (g) NaIO$_4$, RuCl$_3$, aq. MeCN; (h) PPh$_3$, aq. THF.
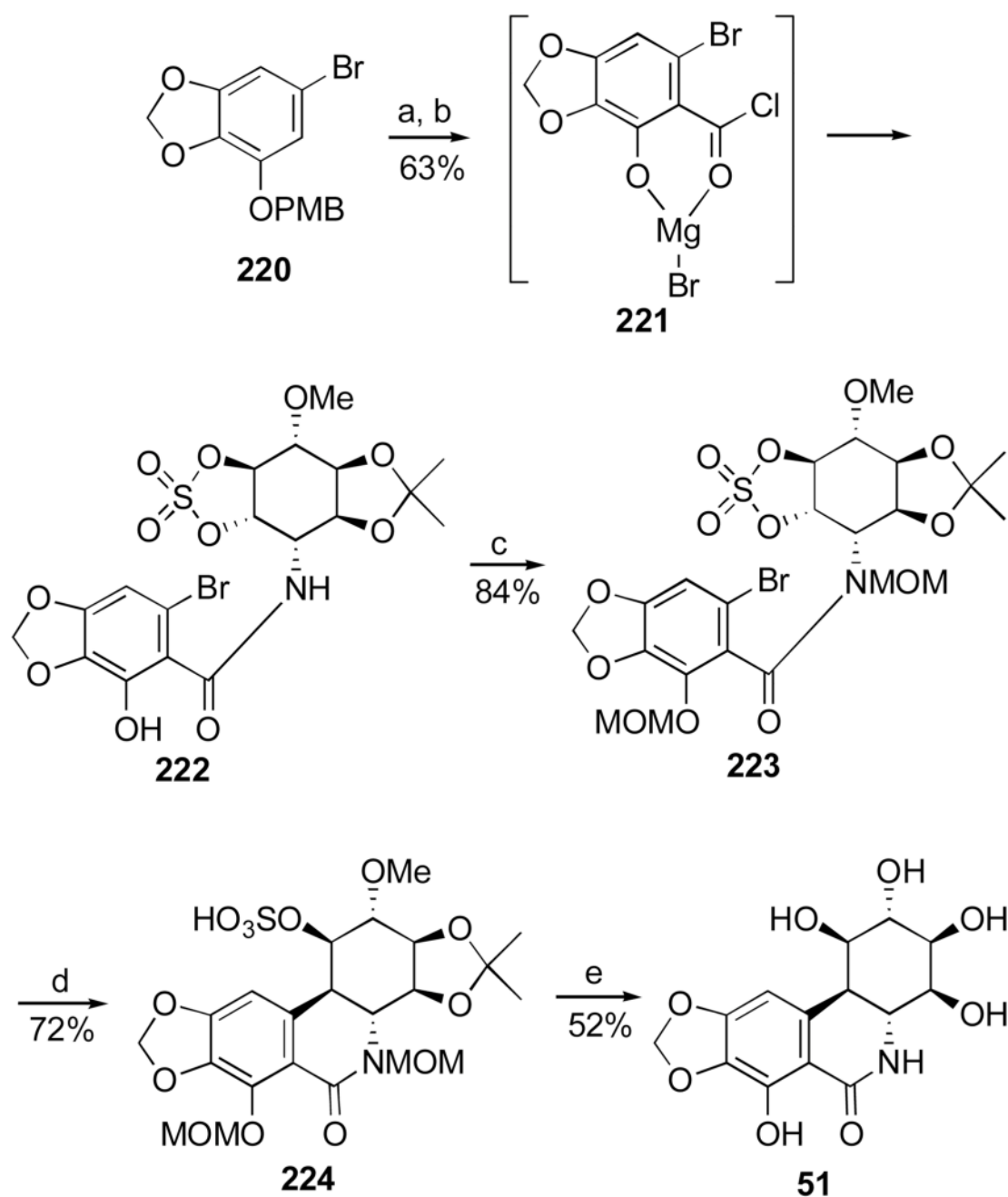
Scheme 20.a.

[a] Reagents and conditions: (a) AcOH, 100 °C; (b) MgBr$_2$·OEt$_2$, COCl$_2$, ether, **219**; (c) K$_2$CO$_3$, MOMCl, DMF; (d) t-BuLi, CeCl$_3$, ultrasound, THF, −78 °C to rt; (e) BBr$_3$, CH$_2$Cl$_2$, −78 °C, then MeOH, −78 °C to rt.

Table 1

Ring types and representative Amaryllidaceae alkaloids*a,b*

	ring type	alkaloid	structure
I	*N*-(3,4-dioxybenzyl)-4-oxyphenethylamine	norbelladine	**1**
II	*N*-(3.4-dioxybenzyl)-3,4-dioxyphenethylamine	rystilline	**2**
III	pyrrolo[de]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 Figure 1 (**1–9**), Figure 2 (**10**), Figure 3 (**18**) and Figure 7 (**43**).

Table 2

The occurrence of narciclasine and its natural congeners in the Amaryllidaceae

compound (structure)	Amaryllidaceae	content mg/kg[a]	ref.
narciclasine (lycoricidinol, **44**)	*Brachystola magna*	1.5	75
	Galanthus nivalis L.	10	15
	G. elwesii Hook	25	"
	Narcissus spp.	30–200	"
	Haemanthus puniceus L.	< 5	"
	H. kalbreyeri	4.4[b]	71
	Hymenocallis littoralis	ND	63
	H. speciosa	2.0	82
	H. expansa	3.0	"
	Leucojum aestivum L.	30	15
	L. Vernum L.	50	"
	Lycoris radiata Herb.	41.6	67
	L. saguinea	ND	69
	N. tazetta L.	65	81
	Pancratium littorale	ND	72
	P. maritimum L.	50	15
	Sprekelia formosissima (L.) Herb	> 5	"
	Sternbergia lutea (L.) Ker-Gawl	< 5	"
	Vallota speciosa L'Her	< 5	"
	Zephyranthes flava	135.0[c]	51
	Z. tubispatha	ND	68
narciprimine (**48**)	*L. sanguinea*	ND	69
	Narcissus spp.	3–5	63
	Z. tubispatha	ND	68
7-deoxynarciclasine (lycoricidine, **45**)	*Lycoris radiata* Herb.	31.9	67
	L. sanguinea	ND	69
	H. littoralis	222.2	70,72
	H. kalbreyeri	1.7[b]	71
trans-dihydronarciclasine (**49**)	*Z. candida*	ND	74
7-deoxy-*trans*-dihydronarciclasine (**50**)	*H. littoralis*	33.0	72
pancratistatin (**51**)	*B. magna*	2.5	75
	H. kalbreyeri	15.8[b]	71
	H. littoralis	144.4	70,72,73

compound (structure)	Amaryllidaceae	content mg/kg[a]	ref.
	H. expansa	4.0	82
	H. pedalis	28.9	"
	H. sonorensis	3.0	"
	H. speciosa	3.0	"
	H. variegata	6.0	"
	P. maritimum	6.0	"
	Z. grandiflora	19.1	80
	Z. carinata	7.5[d]	79
7-deoxypancratistatin (**52**)	*H. kalbreyeri*	2.7[b]	71
kalbreclasine (**46**)	*H. kalbreyeri*	3.1[b]	76
	Z. flava	165.0[c]	51
4-*O*-β-D-glucopyranosylnarciclasine (**47**)	*P. maritimum*	3.3	77
pancratiside (**53**)	*H. kalbreyeri*	19.6[b]	71
telastaside (**54**)	*Polytela gloriosa*	400	78
1-*O*-(3-hydroxybutyryl)pancratistatin (**55**)	*Z. carinata*	23.7[d]	78
1-*O*-(3-*O*-β-D-glucopyranosylbutyryl) pancratistatin (**56**)	*Z. carinata*	43.8[d]	"

[a]Fresh bulbs or insects (*B. magna* and *P. gloriosa*);

[b]Fresh roots;

[c]Fresh seeds;

[d]Air dried bulbs

ND = Not determined

Table 3

Comparative biological evaluation of selected Amaryllidaceae constituents used in the study by Jimenez *et al.*

compound	HeLa cell growth inhibition MIC μM	protein synthesis inhibition IC$_{50}$ μM	DNA synthesis inhibition IC$_{50}$ μM	RNA synthesis inhibition IC$_{50}$ μM
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

Table 4

SAR study of narciclasine derivatives

compound	concentration mM	[^{14}C]Phe incorporation % control	concentration mM	[^{3}H]narciclasine binding % 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	water uptake
	days	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

Table 6

Results of comparative anticancer evaluation of narciclasine and related isocarbostryrils in the NCI sixty cell line screen

compound	mean GI$_{50}$ µM	compare correlation coefficient
narciclasine (**44**)	0.016	0.90
7-deoxynarciclasine (**45**)	0.15	0.90
trans-dihydronarciclasine (**49**)	0.013	0.92
7-Deoxy-*trans*-dihydronarciclasine (**50**)	0.068	0.89
pancratistatin (**51**)	0.091	1
cis-dihydronarciclasine (**77**)	3.8	< 0.6
7-deoxy-*cis*-dihydronarciclasine (**112**)	95.5	< 0.6
isonarciclasine (**78**)	11.8	< 0.6

Table 7

Reported total syntheses of the Amaryllidaceae isocarbostyrils

isocarbostyril	research group	publication year	number of steps[a]	overall yield[a]	reference
(+)-narciclasine	Rigby	1997	23	0.2%	183, 184
(+)-narciclasine	Hudlicky	1999	9	0.6%	185, 146
(+)-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%	109b
(±)-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%	202c
(±)-7-deoxynarciclasine	Ogawa	1991	23	0.04%	203, 204
(+)-7-deoxynarciclasine	Hudlicky	1992	8	12%	205, 206
(±)-7-deoxynarciclasine	Martin	1993	11	4%	207
ent-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

isocarbostyril	research group	publication year	number of steps[a]	overall yield[a]	reference
(+)-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%	213, 189
(+)-7-deoxypancratistatin	Chida	1996	29	0.03%	214
(+)-7-deoxypancratistatin	Keck	1998	15	12%	215
ent-(−)-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%	218, 210
(±)-trans-dihydronarciclasine	Cho	2007	15	11%	219
(±)-7-deoxy-trans-dihydronarciclasine	Tsuda	1978	16	1.1%	220
(+)-7-deoxy-trans-dihydronarciclasine	Chida	1996	24	0.18%	214
(+)-7-deoxy-trans-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 is not commercially available, but had been reported previously by other investigators, the number of steps and yields were taken from the cited sources.

[b] Relay synthesis from (+)-narciclasine.

[c] Improvement of the synthesis by Ohta and Kimoto (Ref. 201).