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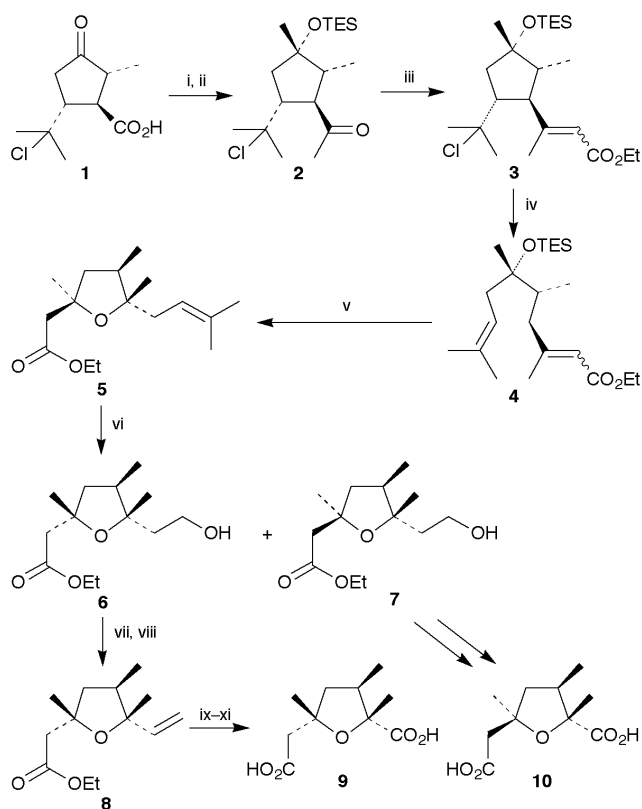
### 1 The synthesis of necines and necic acids

An enantiospecific synthesis of both *cis*- and *trans*-nemorensic acids **9** and **10** has been reported which utilises the regioselective fragmentation of an  $\epsilon$ -halo- $\alpha,\beta$ -unsaturated ester as a key step.<sup>1</sup> The readily available cyclopentanone **1** was treated with methyl-lithium and the major product silylated to provide ketone **2** from which the key  $\epsilon$ -halo- $\alpha,\beta$ -unsaturated esters **3** (*E/Z*  $\geq$  95) were obtained after a Horner–Emmons reaction (Scheme 1). Regioselective samarium iodide promoted fragmentation of **3** gave **4** as a mixture of stereoisomers, which on removal of the silyl group cyclised to a *cis*–*trans* mixture of **5**. Reductive ozonolysis of **5** and chromatographic separation gave alcohols **6** and **7**. Using Grieco's procedure, alcohol **6** was converted into alkene **8**, which following reductive ozonolysis, further oxidation and finally hydrolysis afforded *cis*-nemorensic acid **9**, the necic acid of nemorensine. Similarly, alcohol **7** afforded *trans*-nemorensic acid **10**.

Lactone **11**, a precursor of senecivernic acid and available from earlier work *via* a retro-Diels–Alder approach,<sup>2</sup> has now been incorporated into a stereospecific synthesis of senecivernine **17**.<sup>3</sup> Selective hydrolysis of **11** and reaction with 2-trimethylsilylethanol gave lactone **12** which was carefully ring-opened, silylated, and again selectively hydrolysed to monoacid **13** (Scheme 2). Acid **13** was activated as its acyl phosphate and then condensed with the lithium alkoxide of the protected necine base retronecine **14** to give ester **15**. Selective conversion of the primary hydroxy silyl ether on the necine base to a mesyl group, followed by removal of the trimethylsilylated ester resulted in spontaneous macrolactonisation to **16**. Finally, removal of the protecting group gave senecivernine **17**.

Further details of the synthesis of 7-epiaustraline **18**, reviewed earlier,<sup>4</sup> have been reported, with the physical and spectroscopic data unambiguously confirming that the (–)-isomer had been synthesised.<sup>5</sup> Arising from this synthesis, the natural product previously<sup>6</sup> assigned as (+)-7-epiaustraline is now known to be australine **19**.

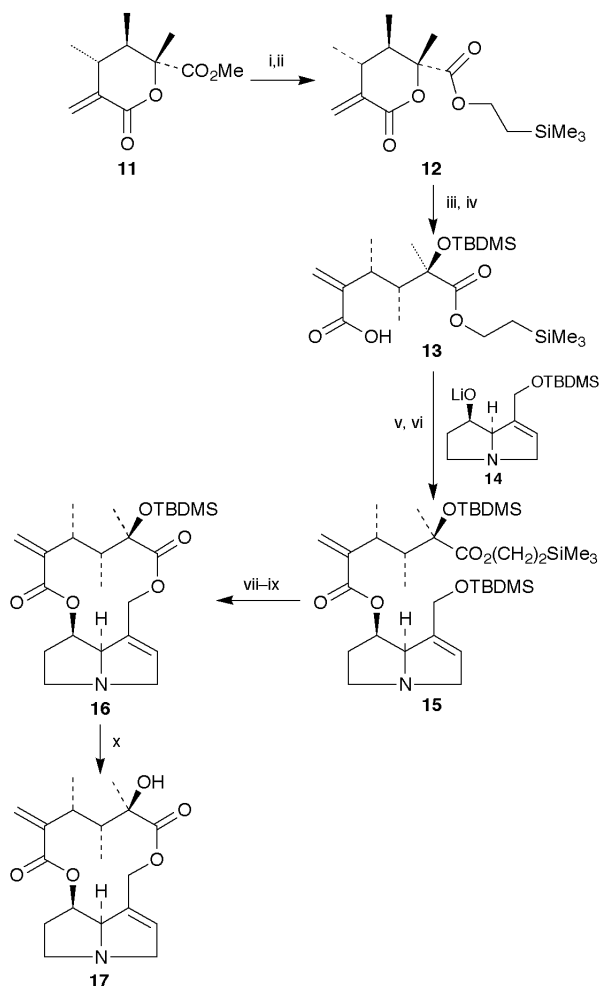
Further work by Montgomery's group on the use of nickel-catalysed reactions has resulted in the synthesis of three indolizidine frog alkaloids and pyrrolizidines **21** and **22**.<sup>7,8</sup> The



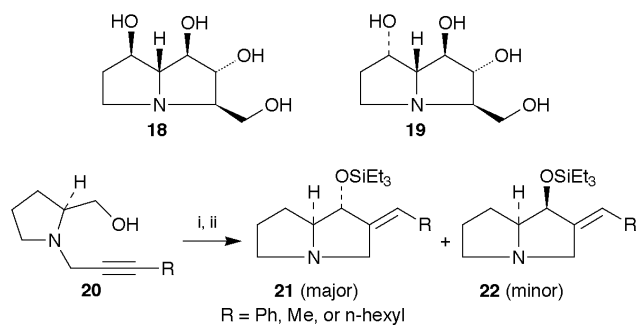
**Scheme 1** Reagents: i, MeLi; ii, TES triflate, 2,6-lutidine; iii, (EtO)<sub>2</sub>OPCH<sub>2</sub>CO<sub>2</sub>Et; iv, SmI<sub>2</sub>, THF-HMPA; v, TBAF; vi, O<sub>3</sub>, then NaBH<sub>4</sub>; vii, (*o*-NO<sub>2</sub>)C<sub>6</sub>H<sub>4</sub>SeCN, Bu<sub>3</sub>P; viii, MCPBA; ix, O<sub>3</sub>, then Ph<sub>3</sub>P; x, NaClO<sub>2</sub>; xi, aq. NaOH, then HCl 1 M.

pyrrolizidines were prepared in two steps from alkynols **20** by Swern oxidation and then cyclisation (Scheme 3).

Four 3,5-disubstituted pyrrolizidines **26–29**, previously isolated from Madagascan frogs,<sup>9</sup> have now been synthesised from a common pyrrolidine precursor **23**.<sup>10</sup> Chain elaboration of **23** by Swern oxidation followed by a Horner–Emmons reaction gave unsaturated ketones **24** and **25** (Scheme 4). Hydrogenation of **24** provided (–)-**26** along with its C-5 epimer as a minor product, while (–)-**27** (and its C-5 epimer as minor product) was obtained from **25** by Wacker oxidation of the terminal alkene, and then hydrogenation. Alternatively, selective reduction of the conjugated alkene of **24** followed by (DHDQ)<sub>2</sub>-PYR ligand-induced Sharpless asymmetric dihydroxylation (AD) provided **30** from which (–)-(6′*S*)-**28** was obtained by selective monotosylation, hydrogenation, and reductive removal of the tosyl group. Replacing the (DHDQ)<sub>2</sub>-ligand in the AD reaction step by the (DHQ)<sub>2</sub>-ligand afforded the (–)-(6′*R*)-isomer of **28**. Similarly, pyrrolizidines (–)-(6′*S*)- and (–)-(6′*R*)-**29** were obtained *via* the identical reaction sequences from **25**.

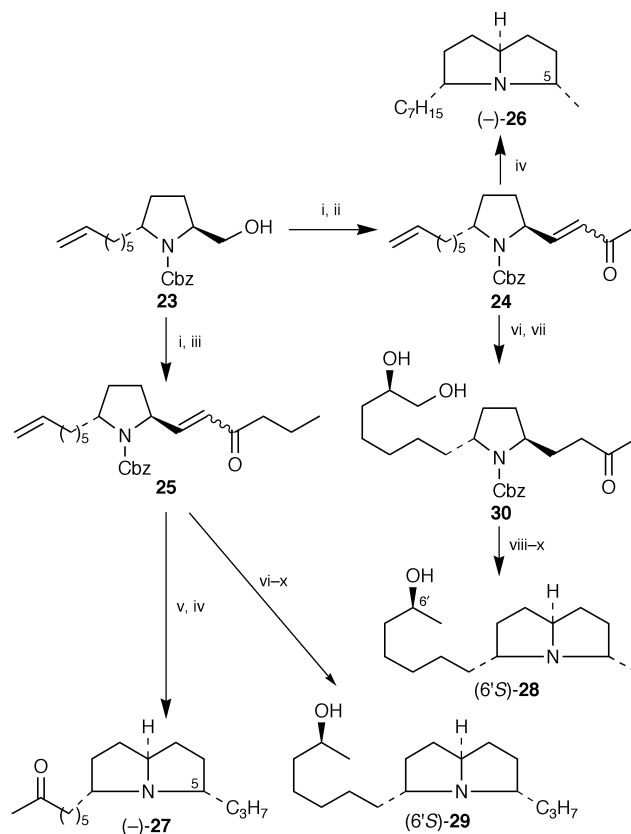


**Scheme 2** Reagents: i, LiOH, THF–H<sub>2</sub>O (1:1), 0 °C, 8 h; ii, DCC, 2-trimethylsilylethanol, DMAP (cat.), CH<sub>2</sub>Cl<sub>2</sub>, 0 °C, overnight; iii, LiOH, H<sub>2</sub>O<sub>2</sub>, THF–H<sub>2</sub>O, 0 °C, overnight; iv, TBDMS triflate, 2,6-lutidine, CH<sub>2</sub>Cl<sub>2</sub>, 0 °C, 1 h, then K<sub>2</sub>CO<sub>3</sub>, MeOH–THF–H<sub>2</sub>O (3:1:1), 25 °C, 3 h; v, Et<sub>3</sub>N, (EtO)<sub>2</sub>P(O)Cl, THF, rt, 3 h; vi, 1.5 equiv. **14**, DMAP (cat.), 1.5 equiv. CH<sub>3</sub>Li, THF, rt, 15 h; vii, 50 equiv. NH<sub>4</sub>F, MeOH–H<sub>2</sub>O (3:1), 60–65 °C, 4 h; viii, MsCl, Et<sub>3</sub>N, CH<sub>2</sub>Cl<sub>2</sub>, 0 °C, 30 min; ix, 14 equiv. *n*-Bu<sub>4</sub>NF, THF, 2 h; x, 40% HF, CH<sub>3</sub>CN, reflux, 6 h.



**Scheme 3** Reagents: i, (COCl)<sub>2</sub>, DMSO, Et<sub>3</sub>N; ii, Et<sub>3</sub>SiH, Ni(COD)<sub>2</sub>, PBu<sub>3</sub>.

In another demonstration of the utility of the tandem [4 + 2]/[3 + 2] nitroalkene cycloaddition methodology, the pentahydroxypyrrolizidine (+)-casuarine **37** has been synthesised by Denmark and Hurd.<sup>11,12</sup> Their synthetic design indicated ketone **35** as the key intermediate, and, after preliminary investigations, this was obtained by [3 + 2] cycloaddition of nitroalkene **31** and benzyloxy vinyl ether (*Z*)-**32** followed immediately by [4 + 2] cycloaddition of the resultant (unstable) nitronate **33** and silyl enone **34** to provide the required **35** as the major diastereomer which was further enriched by preparative HPLC (Scheme 5). Selective reduction of the ketone functionality of **35**, mesylation of the resultant



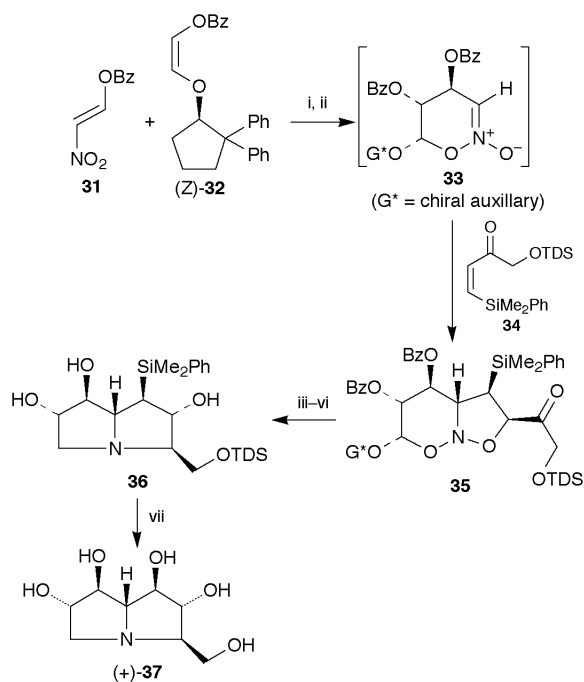
**Scheme 4** Reagents: i, (COCl)<sub>2</sub>, DMSO, Et<sub>3</sub>N; ii, CH<sub>3</sub>COCH<sub>2</sub>PO(OCH<sub>3</sub>)<sub>2</sub>, *i*-Pr<sub>2</sub>EtN, LiCl; iii, *n*-C<sub>7</sub>H<sub>15</sub>COCH<sub>2</sub>PO(OCH<sub>3</sub>)<sub>2</sub>, *i*-Pr<sub>2</sub>EtN, LiCl; iv, H<sub>2</sub>, cat. Pd(OH)<sub>2</sub>; v, O<sub>2</sub>, cat. PdCl<sub>2</sub>, CuCl, DMF, H<sub>2</sub>O; vi, Red-Al, CuBr; vii, AD-mix-β [(DHQD)<sub>2</sub>-PYR ligand] (or AD-mix-α [(DHQ)<sub>2</sub>-PYR ligand]); viii, Bu<sub>2</sub>SnO, then TsCl; ix, H<sub>2</sub>, cat. Pd(OH)<sub>2</sub>; x, Super-Hydride.

10:1 mix of epimeric alcohols and reductive cyclisation with a basic work-up afforded pyrrolizidine **36**. Finally, **36** was converted into the desired (+)-casuarine **37** by mercuric trifluoroacetate dearylation of the silyl group followed by oxidation. The optical rotation of synthetic casuarine is low compared to the natural product, but after checking the enantiomeric purity of the synthetic material the authors concluded that the reported rotation for the natural product is erroneously high.

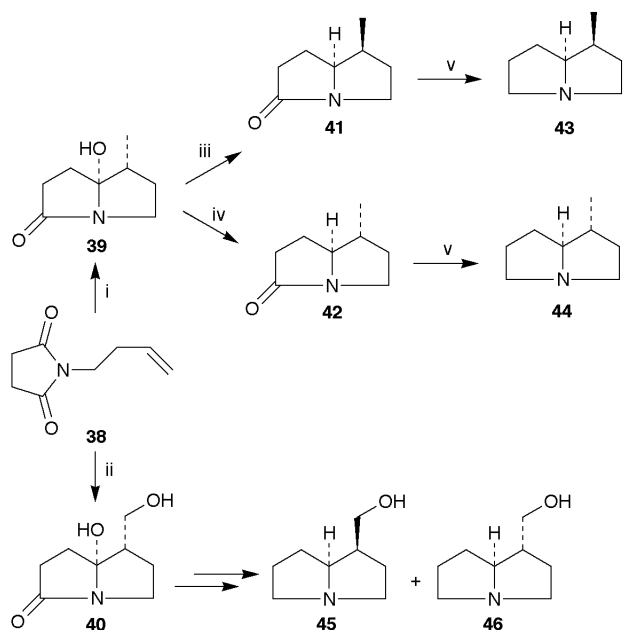
Titanium-mediated cyclisation of ω-vinyl imides has been used by Kim and co-workers to stereoselectively synthesise a number of simple pyrrolizidines (and indolizidines).<sup>13</sup> Treatment of the available ω-vinyl imide **38** with cyclopentylmagnesium chloride in the presence of chlorotitanium trisopropoxide provided on hydrolysis *N*-acylaminol **39**, after simple oxidation and hydrolysis, **40** (Scheme 6). Stereoselective reduction of **39** by catalytic hydrogenation or borohydride reduction afforded either **41** or **42**, which were then further reduced to heliotridane **43** and pseudoheliotridane **44** respectively. In like manner, isoretronecanol **45** and trachelanthamide **46** were obtained from **40**.

As part of their work on antitumour agents, Tepe and Williams have synthesised the derivative **49** which is readily reduced by iron(II)–EDTA to dehydromonocrotaline **50**, a potent DNA alkylating agent.<sup>14</sup> Reaction of monocrotaline, **47**, with fluoren-9-ylmethyl chloroformate in the presence of potassium iodide, oxidation of the resultant allyl iodide and then protection of the aldehyde so formed provided **48** (Scheme 7). The required **49** was obtained by removal of the *N*-protecting group, oxidation of the free amine, and finally removal of the acetal.

The Geissman–Waiss lactone **54**, a key intermediate in a number of necine syntheses, has been prepared from the major

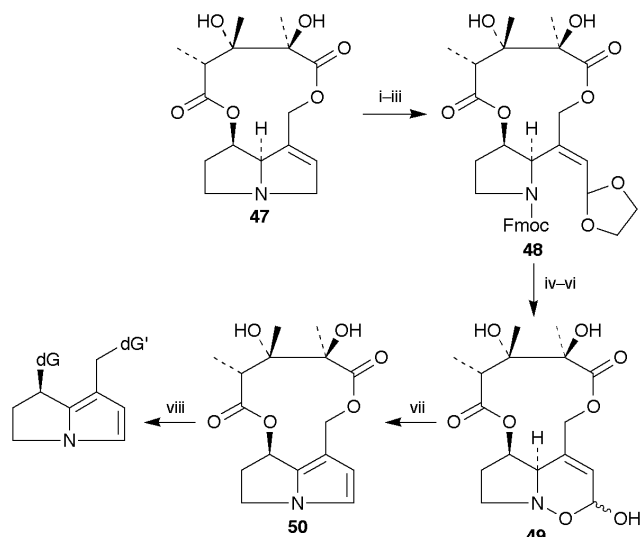


**Scheme 5** Reagents: i,  $\text{SnCl}_4$ , toluene,  $-78^\circ\text{C}$ ; ii,  $\text{Et}_3\text{N}$ , MeOH; iii, L-Selectride, THF,  $-78^\circ\text{C}$ ; iv,  $\text{Ms}_2\text{O}$ , pyridine, 1 h; v, Raney Nickel,  $\text{H}_2$  (260 psi), MeOH; vi,  $\text{K}_2\text{CO}_3$ ; vii,  $\text{Hg}(\text{OTFA})_2$ , TFA, AcOH, 1 h, rt, then  $\text{AcOOH}$ , 18 h, rt.

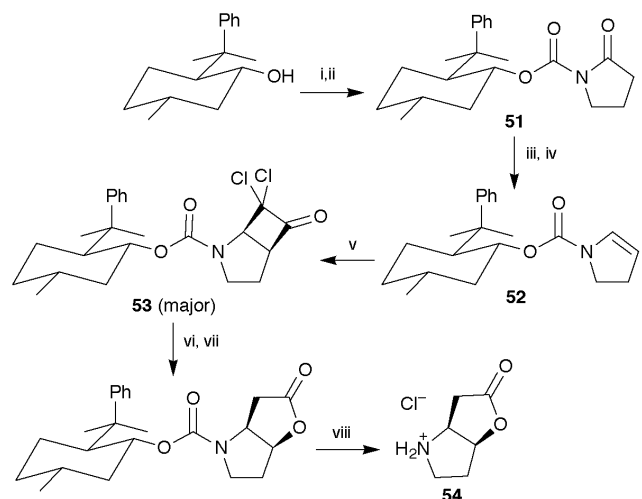


**Scheme 6** Reagents: i,  $(\text{O}-i\text{-Pr})_3\text{TiCl}$ ,  $c\text{-C}_5\text{H}_9\text{MgCl}$ ,  $0^\circ\text{C}$ ; ii,  $(\text{O}-i\text{-Pr})_3\text{-TiCl}$ ,  $c\text{-C}_5\text{H}_9\text{MgCl}$ ,  $0^\circ\text{C}$ , then  $\text{O}_2$ ; iii,  $\text{H}_2$ ,  $\text{PtO}_2$ ,  $\text{EtOAc}$ ,  $\text{CHCl}_3$  (cat.), rt; iv,  $\text{NaBH}_3\text{CN}$ , TFA, MeOH,  $-78^\circ\text{C}$ , then rt; v,  $\text{LiAlH}_4$ , ether, reflux.

diastereomer of carbamate **53** which was used in a study of the diastereoselectivity of [2 + 2] cycloadditions of ketenes to enecarbamates carrying chiral auxiliaries.<sup>15</sup> The starting material (–)-8-phenylmenthol was activated with carbonyl-diimidazole then reacted with pyrrolidin-2-one to provide **51**, which on selective reduction of the ring carbonyl and dehydration provided enecarbamate **52** (Scheme 8). The [2 + 2] cycloaddition reaction of **52** with dichloroketene gave **53** in a 1:4 mixture of diastereomers and, after chromatographic separation, the major isomer was dechlorinated, subjected to a Baeyer–Villiger reaction, and finally the chiral auxiliary removed to give (–)-**54**, isolated as the hydrochloride.



**Scheme 7** Reagents: i, FmocCl, KI, acetonitrile, 10 h, rt; ii,  $\text{AgBF}_4$ , DMSO,  $\text{Et}_3\text{N}$ , rt; iii, TMSCl,  $\text{CH}_2\text{Cl}_2$ ,  $\text{HOCH}_2\text{CH}_2\text{OH}$ ; iv, 50% piperidine,  $\text{CH}_2\text{Cl}_2$ ; v, MCPBA,  $\text{CH}_2\text{Cl}_2$ ; vi, 1% aq. HCl, THF, rt; vii,  $\text{Fe}(\text{II})/\text{EDTA}$ ; viii, DNA.

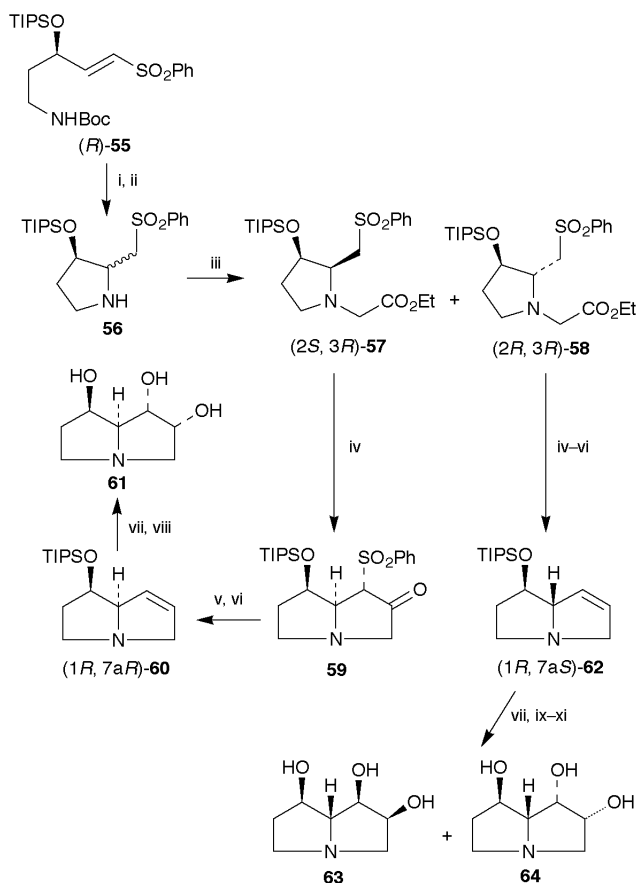


**Scheme 8** Reagents: i, 1,1'-carbonyldiimidazole,  $\text{CH}_2\text{Cl}_2$ , rt; ii, pyrrolidin-2-one,  $85^\circ\text{C}$ , 56 h; iii, DIBAL-H, toluene,  $-78^\circ\text{C}$ ; iv, TFA anhydride, 2,6-lutidine, then reflux 20 min; v, dichloroketene, cyclohexane,  $\text{Et}_3\text{N}$ ,  $40^\circ\text{C}$ ; vi, Zn–Cu,  $\text{NH}_4\text{Cl}$ , MeOH, rt; vii, MCPBA,  $\text{NaHCO}_3$ ,  $\text{CH}_2\text{Cl}_2$ ,  $0^\circ\text{C}$ ; viii, HCl 6 M, reflux.

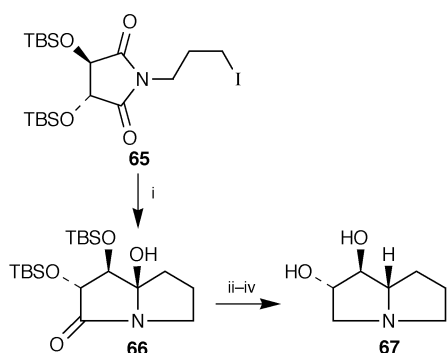
An efficient and stereoselective synthesis of three 1,2,7-trihydropyrrolidines from sulfone **55** has been described.<sup>16</sup> Optically pure (*R*)-**55** was converted to the free amine which cyclised to give pyrrolidines **56** as a 22:78 mix of *cis* and *trans* isomers which were alkylated and separated to provide (2*S*,3*R*)-**57** and (2*R*,3*R*)-**58** (Scheme 9). Intramolecular acylation of (2*S*,3*R*)-**57** afforded unstable **59** which was immediately reduced, and the resultant 70:30 mixture of epimers then converted into (1*R*,7*aR*)-**60** from which pyrrolizidine **61** was obtained as the only product on dihydroxylation and hydrolysis. By an identical reaction sequence, (2*R*,3*R*)-**58** afforded (1*R*,7*aS*)-**62** from which an 80:20 mixture of **63** and **64** was obtained. Separation of the isomers was achieved *via* the triacetates.

Ha and co-workers have used a samarium diiodide catalysed cyclisation of *N*-(3-iodopropyl)imide **65** to provide lactam **66**, which was then converted into pyrrolizidine **67** by removal of the bridgehead hydroxy group, desilylation, and finally reduction of the lactam (Scheme 10).<sup>17</sup>

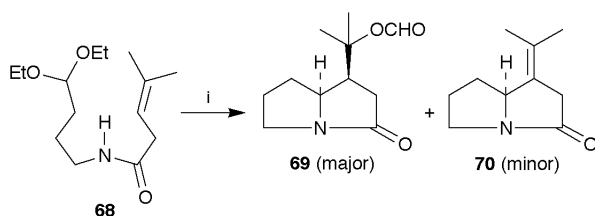
A one-pot biomimetic synthesis of the pyrrolizidine rings **69** and **70** from an acyclic precursor **68**, available from 4-methylpent-3-enoyl chloride and 4-aminobutanal diethyl



**Scheme 9** Reagents: i, TFA,  $\text{CH}_2\text{Cl}_2$ , rt; ii,  $\text{Et}_3\text{N}$ ,  $-78^\circ\text{C}$ ; iii,  $\text{BrCH}_2\text{CO}_2\text{Et}$ ,  $\text{K}_2\text{CO}_3$ ,  $\text{LiI}$  (cat.),  $\text{CH}_3\text{CN}$ ,  $80^\circ\text{C}$ ; iv, LHMDS, THF,  $0^\circ\text{C}$ ; v,  $\text{NaBH}_4$ , MeOH,  $0^\circ\text{C}$ ; vi,  $\text{Na(Hg)}$ ,  $\text{Na}_2\text{HPO}_4$ , MeOH, rt; vii,  $\text{OsO}_4$ ,  $\text{Me}_3\text{NO}$ , acetone– $\text{H}_2\text{O}$  (4:1); viii, 5 M HCl, then Dowex- $\text{OH}^-$ ; ix, 5 M HCl, rt; x,  $\text{Ac}_2\text{O}$ , py, rt; xi,  $\text{NaOH}$ , MeOH– $\text{H}_2\text{O}$ , rt.



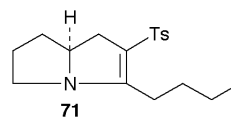
**Scheme 10** Reagents: i,  $\text{SmI}_2$ , tris(dibenzoylmethido)iron(III), THF,  $0^\circ\text{C}$ ; ii, TFA,  $\text{Et}_3\text{SiH}$ ; iii, HCl, MeOH; iv,  $\text{LiAlH}_4$ .



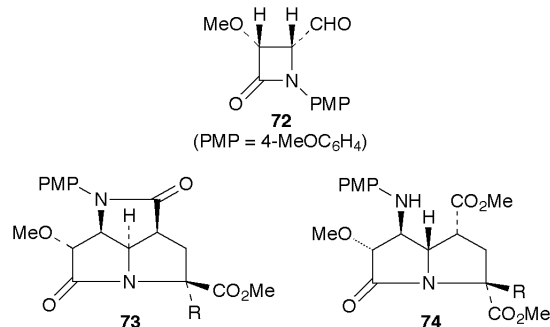
**Scheme 11** Reagents: i, 97%  $\text{HCOOH}$ ,  $25^\circ\text{C}$ , 6 h.

acetal, has been reported by Marson and co-workers (Scheme 11).<sup>18</sup>

Back and Nakajima have reported details of their work on the cyclisations of chloroamines with acetylenic sulfones to provide a variety of nitrogen heterocycles, including pyrrolizidine **71**.<sup>19,20</sup>



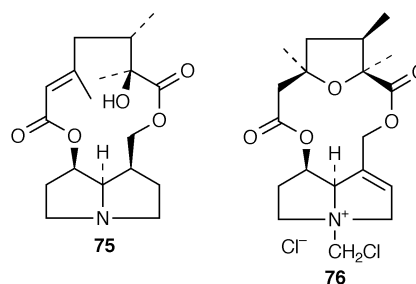
The synthesis of the functionalised bi- and tricyclic pyrrolizidine systems **73** and **74** from chiral  $\beta$ -lactam **72** has been demonstrated by Alcaide and co-workers.<sup>21</sup>



The use of enantiopure pyrroline *N*-oxides for the synthesis of pyrrolizidines and indolizidines has been reviewed,<sup>22</sup> as has the application of allylic derivatives of boron for the synthesis of various alkaloids, including pyrrolizidines.<sup>23</sup>

## 2 Alkaloids of the Asteraceae (Compositae)

A new thirteen-membered macrocyclic pyrrolizidine, iodanthine **75**, has been isolated from the Mexican species *Senecio iodanthus* and *S. bracteatus* along with four known alkaloids (Table 1).<sup>24</sup> Additionally, *N*-chloromethylretroisosenine chloride **76**, a possible artifact of the extraction process, was also obtained from *S. bracteatus*.



Four previously uninvestigated species, *Senecio polypodioides* and *S. runcinatus* from section *Mulgediifolii* and the excluded species *S. madrensis* and *S. prionoapterus*, have been examined for pyrrolizidines as part of a chemotaxonomic study and found to contain six known twelve-membered alkaloids (Table 1).<sup>25</sup> In related work a number of open chain platynecine esters were identified in three further *Senecio* species from the section *Mulgediifolii* (Table 1).<sup>26</sup> A comprehensive study of the variability of the alkaloid content, mainly senecionine and integerrimine, in *Petasites hybridus* growing in Austria has been carried out and has shown marked variation both within and between populations of the plant.<sup>27</sup>

## 3 Alkaloids of the Boraginaceae

Investigation of *Cynoglossum furcatum* has resulted in the isolation and identification of the known alkaloid echinatine and a new alkaloid, neocoramandaline **77**.<sup>28</sup>

Two new pyrrolizidines, ilamine **78** and its *N*-oxide, and the known alkaloids europine and europine *N*-oxide have been isolated from *Heliotropium crassifolium* growing in Ilam, western Iran.<sup>29</sup>

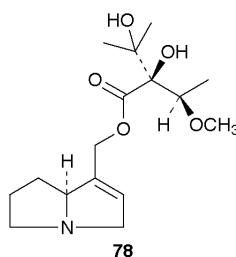
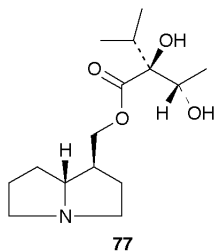
Seven known alkaloids (Table 2), three major and four minor, have been identified in Iranian specimens of *Heliotropium*

**Table 1** Pyrrolizidine alkaloids in the Asteraceae

Species	Pyrrolizidine alkaloids	Ref.
<i>Senecio iodanthus</i>	Iodanthine <sup>a</sup> <b>75</b> , retroisosenine, bulgarsenine, mulgediifoline	24
<i>S. bracteatum</i>	Iodanthine <sup>a</sup> <b>75</b> , retroisosenine, bulgarsenine, mulgediifoline, (1 <i>S</i> )-12-hydroxyretroisosenine, <i>N</i> -chloromethylretroisosenine chloride <b>76</b>	24
<i>S. polypodioides</i>	Platyphylline, platyphylline <i>N</i> -oxide	25
<i>S. runcinatus</i>	Rosmarinine, rosmarinine <i>N</i> -oxide	25
<i>S. madrensis</i>	Platyphylline, platyphylline <i>N</i> -oxide, senecionine	25
<i>S. prionopterus</i>	Senecionine, retrorsine	25
<i>S. doratophyllus</i>	Sarracine, neosarracine, 7 $\beta$ -angelyl-1-methylene-8 $\alpha$ -pyrrolizidine	26
<i>S. deformis</i>	Sarracine <i>N</i> -oxide	26
<i>S. conzantii</i>	Sarracine, neosarracine, 9-angelylplatynecine, 7-tiglylplatynecine	26
<i>S. vulgaris</i>	Retrorsine, seneciphylline, senecionine	52
<i>S. mariettae</i>	Retrorsine	52
<i>S. venosus</i>	Retrorsine	52

<sup>a</sup> New alkaloids.**Table 2** Pyrrolizidine alkaloids in the Boraginaceae

Species	Pyrrolizidine alkaloids	Ref.
<i>Cynoglossum furcatum</i>	Echinatine, neocoromandaline <sup>a</sup> <b>77</b>	28
<i>Heliotropium crassifolium</i>	Europine, europine <i>N</i> -oxide, ilamine <sup>a</sup> <b>78</b> , ilamine <i>N</i> -oxide <sup>a</sup>	29
<i>H. europaeum</i>	Europine, heliotrine, heliotrine <i>N</i> -oxide, lasiocarpine, lasiocarpine <i>N</i> -oxide, acetylasiocarpine, acetylasiocarpine <i>N</i> -oxide	30
<i>Echium rawolfii</i>	Echimidine, 7-angeloylretronecine, 7-tigloylretronecine, 7-angeloyl-9-(2-methylbutyryl)retronecine, 7-tigloyl-9-(2-methylbutyryl)retronecine, 7-acetyllycopsamine, 7-angeloyl-9-(2,3-dihydroxybutyryl)retronecine, 7-tigloyl-9-(2,3-dihydroxybutyryl)retronecine, 7-angeloyllycopsamine, 7-tigloyllycopsamine, echimidine isomer (tigloyl), uplandicine (trace)	31
<i>E. horridum</i>	Echimidine, 7-angeloylretronecine, 7-tigloylretronecine (trace), 7-angeloyl-9-(2-methylbutyryl)retronecine, 7-tigloyl-9-(2-methylbutyryl)retronecine, lycopsamine, 7-acetyllycopsamine, 7-angeloyl-9-(2,3-dihydroxybutyryl)retronecine, 7-tigloyl-9-(2,3-dihydroxybutyryl)retronecine, 7-angeloyllycopsamine, 7-tigloyllycopsamine, echimidine isomer (tigloyl), uplandicine (trace)	31

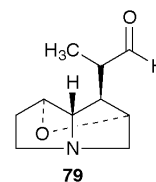
<sup>a</sup> New alkaloids.

*europaeum* L. population Garmsar.<sup>30</sup> The known alkaloid echimidine has been isolated from *Echium rawolfii* and *E. horridum*, and a further twelve minor pyrrolizidines identified, either as the free base or as the *N*-oxide, by GC and GC-MS analysis (Table 2).<sup>31</sup> Both species had similar alkaloid profiles.

#### 4 Alkaloids of the Convolvulaceae

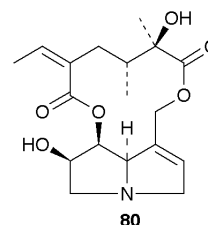
*N*-Formylloline **79** has been isolated from *Argyrea mollis* in which it was present in both the aerial vegetative parts and the roots.<sup>32</sup> GC-MS analysis showed that loline, *N*-methylloine and *N*-propionylnorloline (decorticasine) were also present. Lolines were absent from three other *Argyrea* species and numerous

other species from fourteen genera in the Convolvulaceae. This is the first reported occurrence of lolines in the Convolvulaceae.



#### 5 Alkaloids of the Fabaceae

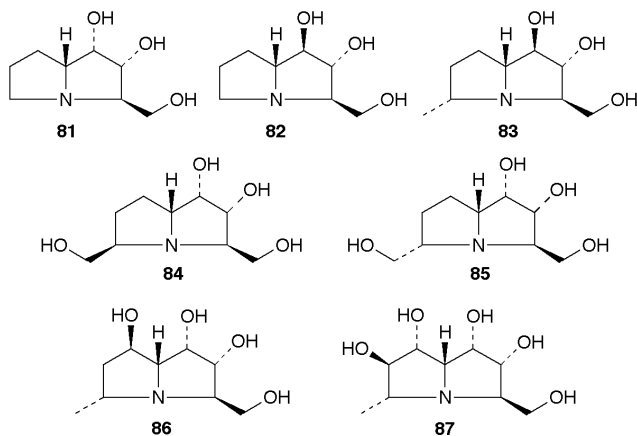
The known alkaloid anacrotine **80** has been isolated from seeds of *Crotalaria trifoliastrum*, the roots of which are used in traditional medicine.<sup>33</sup>



#### 6 Alkaloids of the Hyacinthaceae

New sources of pyrrolizidines have been identified with the discovery of a number of novel polyhydroxypyrrolizidines in investigations of *Hyacinthoides non-scripta*, *Scilla campanulata* and *Muscari armeniacum*. Hyacinthacines A<sub>1</sub>–A<sub>3</sub>, B<sub>3</sub> and C<sub>1</sub> (**81**–**83**, **86** and **87** respectively) were isolated from *M. armeniacum*,<sup>34</sup> while *H. non-scripta* was found to contain hyacinthacines

B<sub>1</sub> and C<sub>1</sub> (**84** and **87**) and *S. campanulata* contained hyacinthacines B<sub>1</sub> and B<sub>2</sub> (**84** and **85**).<sup>35</sup>

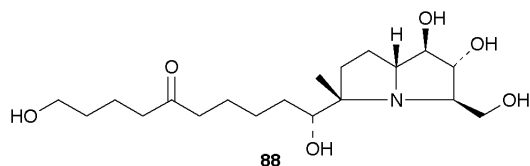


## 7 Alkaloids of the Labiatae

An investigation of *Ajuga parviflora* for bioactive compounds has revealed the presence of the known toxic alkaloids senecionine and integerrimine as well as two new antifungal withanolides.<sup>36</sup>

## 8 Alkaloids of the Moraceae

During investigations into the active components of crude drugs obtained from the branches of the tree *Broussonetia kazinoki* a new pyrrolizidine alkaloid broussonetine N (**88**), which has inhibitory activities against several  $\beta$ -glycosidases, was isolated.<sup>37</sup> This is the first report of pyrrolizidines from the Moraceae.



## 9 Alkaloids in animals

Studies on eleven species of *Longitarsus* flea beetles have shown that all eleven species sequestered pyrrolizidine alkaloids obtained from their host plants. Some degradation of complex plant alkaloids to simpler structures such as the necine bases was also observed in all species.<sup>38,39</sup> A study of the dietary sources of alkaloids present in poison frogs (*Dendrobates auratus*) has shown that some of the alkaloids, including *cis*- and *trans*-3,5-dialkylpyrrolizidines of mass 251 Da are obtained from species of myrmicine ants.<sup>40</sup> The moth *Utetheisa ornatrix* is known to sequester pyrrolizidine alkaloids obtained from its larval food plants. A study has now shown that incorporation of pyrrolizidines into the eggs provides defence against predation by larvae of the green lacewing, a natural predator.<sup>41</sup> The deterrent effect is greatest for the *N*-oxide form of the alkaloids, and the principal alkaloid in the eggs is monocrotaline *N*-oxide. New records have been reported for two insect species, *Largus rufipennis* (Hemiptera) and *Chauliognathus fallax* (Coleoptera), feeding on and sequestering the pyrrolizidine alkaloids senecionine, integerrimine, retrorsine and usaramine from *Senecio braziliensis*.<sup>42</sup> Both species are warningly coloured and rejected by predators.

The pyrrolizidine alkaloid and nitrogen concentration in leaves of *Senecio jacobaea* has been found to decrease with leaf age, and the within-plant distribution of greatest feeding damage by the generalist herbivores *Spodoptera exigua* Hubner and *Mamestra brassicae* L. correlates well with regions of lowest alkaloid concentration. This implied that the herbivores were forced to feed on sub-optimal parts of the plant, which

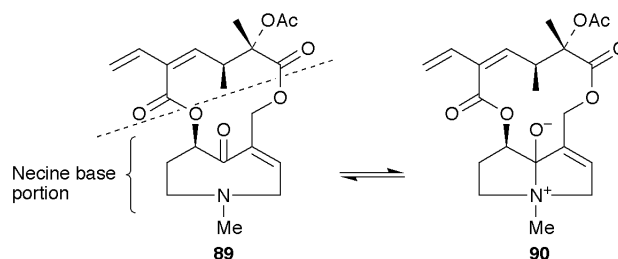
minimised plant nutrient losses.<sup>43</sup> *Senecio jacobaea*, a host-plant of the cinnabar moth *Tyria jacobaea*, has two chemotypes which contain jacobine or erucifoline as major components. *Senecio erucifolius*, which also contains erucifoline, is not recorded as a host-plant for the cinnabar moth. Both oviposition and larval feeding experiments have shown that the cinnabar moth does not discriminate between *Senecio erucifolius* and the two chemotypes of *Senecio jacobaea*, and thus factors other than erucifoline content must be determining the choice of host plant. Flowering timing has been suggested as a factor.<sup>44</sup>

A study of two *Oreina* species of leaf beetle, *O. cacaliae* and *O. speciosissima* has shown that adults of both species have the same pyrrolizidine storage capacity, but that in larvae, *O. cacaliae* has the higher capacity. Additionally, all tissues of the larvae of *O. cacaliae* were found to contain pyrrolizidines, with the greatest concentration (50–60%) being in the hemolymph.<sup>45</sup> Using tracer feeding experiments the biochemical mechanisms of pyrrolizidine alkaloid sequestration in the alkaloid-adapted leaf beetle *Oreina cacaliae* have been investigated. Alkaloid *N*-oxides were found to be sequestered unchanged, but free bases were sequestered as the *O*-glycoside. A mechanism to account for the observations has been proposed.<sup>46</sup>

Possible cases of horses suffering from pyrrolizidine alkaloid poisoning resulting from the consumption of young pasture containing houndstongue (*Cynoglossum officinale*) has been reported.<sup>47</sup> Significant alterations in bovine serum biochemistry profiles associated with prolonged consumption of tall fescue (*Festuca arundinacea*) infected with the endophytic fungus *Neotyphodium coenophialum* compared to uninfected tall fescue have been reported.<sup>48</sup> Feeding trials have shown that in sheep there does not appear to be any increased susceptibility to hepatotoxicity arising from simultaneous consumption of ergot alkaloids in endophyte-infected tall fescue and pyrrolizidine alkaloids in tansy ragwort (*Senecio jacobaea*).<sup>49</sup> A review of physical and chemical contaminants (including pyrrolizidine alkaloids) in grains used in livestock feeds has been published.<sup>50</sup>

## 10 General studies

The unusual solubility of the hepatotoxic otonecine type alkaloids clivorine and ligularine in both aqueous and non-polar organic solvents has been investigated by NMR spectroscopy.<sup>51</sup> For both alkaloids the necine base portion in non-polar solvents was found to exist as a non-ionised monocyclic ring, **89** (for clivorine), while in polar solvents the necine base existed as an ionised bicyclic structure, **90**.



A new method for the detection and identification of pyrrolizidines in crude plant extracts using HPLC/MS and HPLC/<sup>1</sup>H NMR has been developed.<sup>52</sup> Both on-flow and stopped-flow techniques were used to obtain NMR spectra, with the stopped-flow technique permitting the acquisition of <sup>1</sup>H–<sup>1</sup>H COSY 2D spectra. The method was tested by detecting the known alkaloids retrorsine, seneciophylline and senecionine in *Senecio vulgaris*, and retrorsine as the major alkaloid in the previously uninvestigated species *S. mariettae* and *S. venosus*. The use of ammonia chemical ionisation tandem mass spectrometry as a means of structure determination for a

variety of alkaloid types, including pyrrolizidines, has been described and applied to extracts from amphibian skin.<sup>53</sup> The analytical methods used in the isolation, purification, and quantification (but not structural elucidation) of pyrrolizidine alkaloids in plants, foodstuffs, and other biological materials have been described.<sup>54</sup>

### 11 Pharmacological and biological studies

Pyrrolizidine poisoning of a number of children by South African traditional medicines has been verified in a clinical study.<sup>55</sup> Pretreatment of rats with glycyrrhizin and glycyrrhetic acid, the major biologically active ingredients of liquorice, significantly reduced retrorsine-induced liver damage.<sup>56</sup> A study to identify the specific cytochrome P 450 (CYP) enzymes involved in retrorsine metabolism has found that one or more of CYPs 1A1, 1A2, 2E1 and 2B1/2 may be involved.<sup>57</sup> A method for the detection of dehydroretronecine-modified DNA adducts using <sup>32</sup>P-post-labelling/HPLC has been developed.<sup>58</sup> A comparative study of DNA-crosslinking by activated pyrrolizidine alkaloids has been carried out, and the structural features most likely to confer potent cross-linking activity identified.<sup>59</sup> A comprehensive review of the occurrence, biological activity and potential therapeutic application of alkaloids which are sugar-mimic glucosidase inhibitors has been published. Included in the structures discussed are the alexine, australine and hyacinthacine pyrrolizidine types.<sup>60</sup> Other reviews include: the food safety implications of pyrrolizidines being transferred into eggs and other human foodstuffs via contaminated animal feeds;<sup>61</sup> DNA minor groove alkylating agents, including pyrrolizidine alkaloids;<sup>62</sup> biochemical/medical aspects of pyrrolizidines in the human diet;<sup>63</sup> toxicity within the Asteraceae with emphasis on pharmaceutically important species (in Czech);<sup>64</sup> and a review (in Chinese) on pharmaceutical studies of pyrrolizidine alkaloids.<sup>65</sup>

### 12 Acknowledgements

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