

REVIEW ARTICLE

The History of the Glycosidase Inhibiting Hyacinthacine C-type Alkaloids: From Discovery to Synthesis

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Abstract: Background: The inherent glycosidase inhibitory activity and potentially therapeutic value of the polyhydroxylated pyrrolizidine alkaloids containing a hydroxymethyl substituent at the C-3 position have been well documented. Belonging to this class, the naturally occurring hyacinthacine C-type alkaloids are of general interest among iminosugar researchers. Their selective micromolar α -glycosidase inhibitory ranges (10 – 100 μ M) suggest that these azasugars are potential leads for treating type II diabetes. However, the structures of hyacinthacine C1, C3 and C4 are insecure with hyacinthacine C5 being recently corrected.

Objective: This review presents the hyacinthacine C-type alkaloids: their first discovery to the most recent advancements on the structures, biological activities and total synthesis.

Conclusion: The hyacinthacine C-type alkaloids are of exponentially increasing interest and will undoubtedly continue to be reported as synthetic targets. They represent a challenging but rewarding synthetic feat for the community of those interested in accessing biologically active iminosugars. Since 2009, ten total syntheses have been employed towards accessing similarly related products but only three have assessed the glycosidase inhibitory activity of the final products. This suggests the need for an accessible and universal glycosidase inhibitory assay so to accurately determine the structure-activity relationship of how the hyacinthacine C-type alkaloids inhibit specific glycosidases. Confirming the correct structures of the hyacinthacine C-type alkaloids as well as accessing various analogues continues to strengthen the foundation towards a marketable treatment for type II diabetes and other glycosidase related illnesses.

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1. PYRROLIZIDINE ALKALOIDS

Pyrrolizidine alkaloids, particularly those that contain a hydroxymethyl substituent at C-1 (Fig. 1), have been of exponentially growing interest since the 1960s [1].

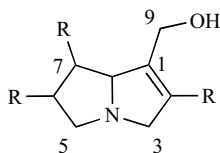


Fig. (1). General structure for pyrrolizidine alkaloids.

Their appreciation is largely a result of the increasing number of deaths to livestock that has consumed plants rich in pyrrolizidine alkaloids [1]. A memorable example occurred in Australia in the late 1970s, whereby a large number of chicken poisonings were reported [2]. On closer inspection, researchers found that the ill-thrift, ascites and degenerative lesions on the chickens, were the result of a commercial poultry feed containing heliotrine and lasiocarpine (Fig. 2): pyrrolizidine alkaloids found in the seeds of *Heliotropium stenophyllum* [2]. However, it is more alarming that

many cases of liver disease and cancer in humans have been the direct result of pyrrolizidine alkaloid consumption [3-6]. This widespread occurrence is not surprising since there have been recorded findings of pyrrolizidine alkaloids detected in wheat flour, honey and also cow's milk [7-9]. At least from an economic point of view, it remains important that improved methods of detection, chromatographic separation and chiefly, natural product characterization are continually explored. In the early 1980s, pyrrolizidine alkaloids were deemed pernicious upon ingestion and were consequently flagged as a world-wide health concern [1]. Their toxicity is a result of the 1,2-unsaturated bond, in combination with an ester at the C-9 position [1]. After consumption, hepatic mixed function oxidases convert these pyrrolizidine alkaloids to their counter pyrrole derivatives. These metabolic products are highly reactive and can scar various organs in the body, particularly rich in thiol containing amino acids [10, 11].

During this time, only a few examples existed of pyrrolizidine alkaloids that displayed anti-tumor [12] and neuromuscular blocking [13] activities and so this class of alkaloid was generally thought to be toxic. However, by the late 1980s, this view drastically changed after the discovery of the polyhydroxylated pyrrolizidine alkaloids containing a hydroxymethyl substituent at the C-3 position [14]. Alexine (Fig. 3) was the first compound of this type to be isolated from natural sources, more specifically, the legume *Alexia leiopetala* [14]. Its structure was unequivocally confirmed by X-ray crystallography [14] and just a few months

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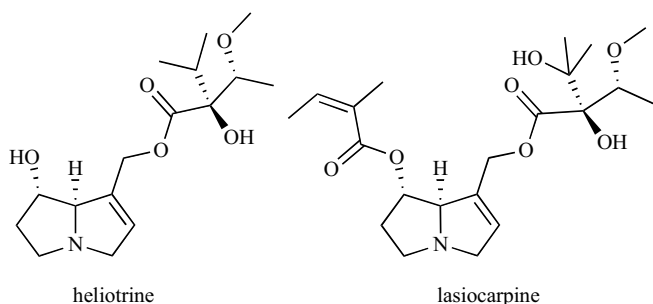


Fig. (2). Pyrrolizidine alkaloids heliotrine and lasiocarpine.

later, its C-7a epimer (also crystalline), australine, was reported as a constituent of *Castanospermum australe* seeds (Fig. 3) [15]. Following these discoveries, a number of closely related epimers were also isolated from *C. austral* [16-19]. Interestingly, alexine and australine along with their related epimers displayed notable anti-viral properties as well as low IC₅₀ values towards specific glycosidases [14, 15]. These findings attracted significant attention to the polyhydroxylated pyrrolizidine alkaloids, as contrary to their non-hydroxylated analogues, they proved to be medicinally useful.

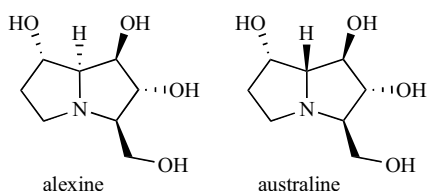


Fig. (3). Polyhydroxylated pyrrolizidine alkaloids containing a hydroxymethyl substituent at C-3, alexine and its C-7a epimer australine.

The polyhydroxylated pyrrolizidine alkaloids unique structure and high oxidation level give rise to their reported anti-viral properties and other medicinal applications such as treatment for diabetes, obesity and cancer [20, 21]. Their affinity for glycosidases is a result of their mimicry of pyranosyl and furanosyl moieties of carbohydrates. The strength and specific inhibition is largely dependent on the shape, size and stereoconfiguration as well as the target glycosidase. Before the discovery of alexine and australine, it was generally accepted that glycosidase inhibitors were restricted in Nature and so the search for related analogues became of increasing interest for medicinal chemists [15, 22-25].

It was, therefore, an exciting discovery when the first pentahydroxylated pyrrolizidine alkaloid, casuarine, was isolated from the bark of *Casuarina equisetifolia* L (Fig. 4) reported by Nash *et al.* in 1994 [26]. Nash and colleagues were initially interested in the bark of *C. equisetifolia* L because of its traditional use as a medicine to treat diarrhoea, dysentery and colic [26, 27]. Perhaps a more convincing reason for Nash *et al.* to examine this natural source, was due to previous reports of prescribed *C. equisetifolia* L bark extract for the treatment of breast cancer in Western Samoa [26]. Their investigation started with a GC-MS analysis of the extract, where a pentahydroxy- nitrogen-containing species, later established to be casuarine, was identified to be one of the major compounds present. Moreover, it contained the highest oxidation level to be found in any related naturally occurring iminosugar. Its analogous structure to alexine and australine led researchers to suspect it contained inherent glycosidase inhibitory activity. This was confirmed to be correct after various glycosidase inhibition tests [26].

Casuarine has also been identified as a common constituent within the leaves and seeds of *Eugenia jambolana*, a plant native to India known particularly for its various medicinal uses including the management of diabetes and bacterial infections [28]. Although

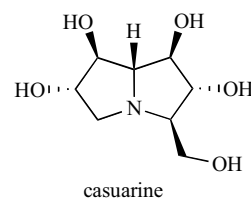


Fig. (4). Polyhydroxylated pyrrolizidine alkaloid casuarine.

initially labelled as uniflorine A and B, it was later corrected that casuarine along with its C-6 epimer was the biologically active constituents isolated in 2000 from the leaves of a similar species *Eugenia uniflora* [29-35] (Fig. 5). The only other naturally occurring casuarine epimer reported in the literature is the C-3 epimer which was isolated from *Myrtus communis* L. in 2006 by Fleet *et al.* [36]. With respect to casuarine, its notably weaker inhibition of α -D-glucoside is a result of its single stereochemical difference [36].

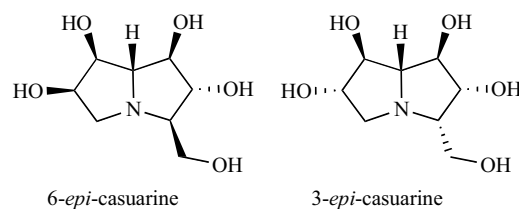


Fig. (5). Casuarine derivatives 6-epi-casuarine and 3-epi-casuarine.

2. HYACINTHACINE ALKALOIDS

One recently discovered subclass of polyhydroxylated pyrrolizidine alkaloid that has attracted considerable attention for their inherent glycosidase inhibitory activities is the hyacinthacine alkaloids. Despite their analogous structure to casuarine, alexine and australine, these alkaloids have been named after their original source, namely the Hyacinthaceae subfamily within the Asparagaceae [37]. As shown in Fig. (6), the hyacinthacine alkaloids can be divided into three classes: Hyacinthacine A, B and C; whereby the letter assignment denotes whether 0, 1 or 2 hydroxy/hydroxymethyl substituents are found on the B-ring of the alkaloid, respectively.

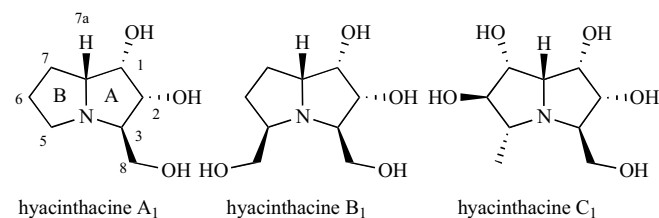


Fig. (6). Hyacinthacines A₁, B₁, C₁, containing 0, 1 and 2 hydroxy or hydroxymethyl substituents on the B-ring of the pyrrolizidine, respectively.

The discovery of the hyacinthacine alkaloids can be traced back to the late 1960s and is considered an unprecedented finding, largely because Asparagaceae is unrelated to the Leguminosae, Casuarinaceae and Myrtaceae plant families [38]. In 1967, a report from *The Veterinary Record* written by Thursby-Pelham, detailed the toxicological effects on livestock after consuming *Hyacinthoides non-scripta* [39]. More specifically, Thursby-Pelham found that ingestion led to abdominal pain and dysentery in horses and lethargy and dullness in cows [39]. The plants metabolites, however, remained a mystery until 1997, where, prompted by this report and perhaps also the availability of this material, Fleet *et al.* identified the presence of five pyrrolizidine alkaloids present in the ethanolic fraction of the leaves [40]. This was the first report of any

such alkaloids from the Hyacinthaceae species and although the plant was considered detrimental to livestock health, Fleet *et al.* were able to demonstrate that the isolated pyrrolidine alkaloids were potential therapeutic leads. Glycosidase inhibition studies of these isolates found that they might serve as useful chemotherapeutic agents against cancers, viruses and also diabetes [40].

2.1. Hyacinthacine Isolation and Biological Activities

Two years after this novel finding, several of the same authors led by Asano, reported their investigation into the fruits and stalks of the *H. non-scripta* [41]. To further explore the natural abundance of similar compounds, their research also detailed the analysis of the bulbs of a related species, *Scilla campanulata* [41].

In addition to the isolation of six pyrrolidine alkaloids, three novel polyhydroxylated pyrrolizidine alkaloids labelled as hyacinthacines were reported [41]. More specifically, from the 50% ethanoic extracts of the *H. scripta* stalks, hyacinthacine C₁ was isolated, and from the 50% ethanoic extract of the *S. campanulata* bulbs, hyacinthacine B₂ was isolated [41]. Hyacinthacine B₁ was common in both plants and thus found as a constituent in both extracts [41]. Although not necessarily potent, these three alkaloids gained considerable interest because of their selective glycosidase inhibition. Hyacinthacine B₁ and B₂ both displayed weak inhibition of almond β -glucosidase (IC₅₀ values of 320 μ M and 100 μ M, respectively) and bovine liver β -galactosidase (IC₅₀ values of 110 μ M and 160 μ M, respectively). Both alkaloids also showed selective inhibition for rat intestinal lactase β -galactosidase, however, hyacinthacine B₂ proved far more active (IC₅₀ = 3.6 μ M) than hyacinthacine B₁ (IC₅₀ = 270 μ M). Interestingly, hyacinthacine C₁ proved moderately active against the amyloglucosidase of *Aspergillus niger* (IC₅₀ = 84 μ M), whereas hyacinthacine B₁ and B₂ displayed no inhibition for these two glycosidases.

Given these noteworthy glycosidase inhibitory activities, the therapeutic potential warranted further investigation of the hyacinthacine alkaloids. Since there had not yet been a proven synthetic route to access these novel structures, natural product investigation was the only viable option. To increase the chance of finding similar novel hyacinthacine alkaloids, the same authors reported in 2000, their investigation into the bulbs of the related *Muscari armeniacum* whereby they identified four novel hyacinthacine type alkaloids [42]. More specifically, Asano and co-workers successfully isolated (from 60% ethanoic extracts) hyacinthacine C₁ as well as new Hyacinthacines A₁, A₂, A₃ and B₃ [42]. When tested against a panel of glycosidases, hyacinthacines A₁, A₂, A₃ and B₃ displayed weak to moderate activity against rat intestinal lactase β -galactosidase (IC₅₀ values of 4.4 μ M, 73 μ M, 160 μ M and 18 μ M, respectively) and also *A. niger* amyloglucosidase (IC₅₀ values of 25 μ M, 8.6 μ M, 17 μ M and 51 μ M, respectively). Hyacinthacine A₁ proved to be the only alkaloid active against rat epididymis α -L-fucosidase (IC₅₀ = 46 μ M) and also rice α -glucosidase (IC₅₀ = 240 μ M). Hyacinthacine A₁ and A₂ also displayed activity against almond β -glucosidase with IC₅₀ values of 250 μ M and 150 μ M, respectively [42].

Despite what could be considered a small compound library, Asano and co-workers remarked on the difficulty to predict the specificity and potency of glycosidase inhibition, based on absolute configuration and also the degree of substitution on the pyrrolizidine ring [42]. Concluding statements from this body of work highlighted the need for a variety of highly oxygenated or substituted pyrrolizidine alkaloids to better understand the structural requirements for glycosidase inhibition [42]. Concurrently, glycosidase inhibitors were also receiving considerable attention as therapeutic agents. In addition to potential treatment for NIDDM (type II diabetes), glycosidase inhibitors were also established as

possible treatments for tumor metastasis, viral infections, and various lysosomal storage disorders [20, 43-47]. For these reasons, an investigation into the hyacinthacine type alkaloids also received increased attention and resulted in perhaps the most profitable recordings of novel hyacinthacine alkaloids isolated from a single species to date by Asano *et al.* in 2002 [48]. Their report detailed the analysis of the bulbs of the related *Scilla sibirica* that were originally purchased from a flower shop in 1999. Specifics of this report include 9 kg of *S. sibirica* bulbs being homogenized in 60% aqueous ethanol, and further extracted to give seven new hyacinthacine alkaloids appropriately named hyacinthacines A₄, A₅, A₆, A₇, B₄, B₅ and B₆ [48] (Table 1). Although the majority of these hyacinthacine alkaloids proved relatively inactive towards the selected panel of glycosidases, they still added to the understanding of the structure-activity relationships of these and related compounds. In summary, the only active hyacinthacine alkaloids were A₅, B₄, and B₅, where they showed inhibitory activity towards the amyloglucosidase of *A. niger* with IC₅₀ values of 110 μ M, 89 μ M and 110 μ M, respectively. In addition to this, hyacinthacine B₄ displayed a moderate inhibition of bovine epididymis α -L-fucosidase with an IC₅₀ value of 23 μ M [48].

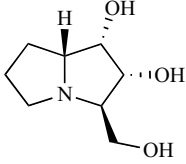
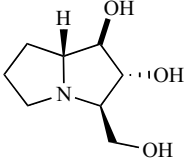
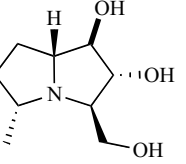
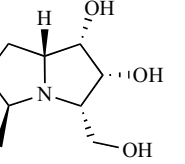
Two years later, Asano and co-workers reported findings of long side chain pyrrolizidines and pyrrolizidines in a closely related Asparagaceae, *Scilla peruviana* [49]. Asano *et al.* isolated three novel hyacinthacine A₁-type related alkaloids along with one novel australine type related alkaloid [49]. These long-chain hyacinthacine derivatives were isolated from 5 kg of the bulbs using 50% aqueous ethanoic extracts and were accordingly named α -5-C-(3-hydroxybutyl)hyacinthacine A₁, α -5-C-(1,3-dihydroxybutyl)hyacinthacine A₁, α -5-C-(1,3,4-trihydroxybutyl)hyacinthacine A₁ and α -5-C-(3-hydroxybutyl)-7-*epi*-australine [49]. When tested against a panel of glycosidases, α -5-C-(1,3-dihydroxybutyl)hyacinthacine A₁ and α -5-C-(3-hydroxybutyl)-7-*epi*-australine displayed relatively good activity against yeast α -glucosidase (IC₅₀ values of 3.6 μ M and 6.6 μ M, respectively), however only the former alkaloid, along with α -5-C-(1,3,4-trihydroxybutyl)hyacinthacine A₁, proved relatively low inhibition against almond β -glucosidase and *C. saccharolyticum* β -glucosidase with IC₅₀ values of 9.5 μ M, 25.4 μ M and 5.1 μ M, 11.4 μ M, respectively [49].

The most recent addition to the naturally occurring hyacinthacine alkaloids is the 2007 reporting of six new isolates from *Scilla socialis* [50]. Kato and co-workers initially purchased plants of *S. socialis* in 2003 and cultivated this species in a heated greenhouse. Once grown, 2.3 kg of the plant bulbs were homogenized in a 50% ethanol solution and further investigated whereby α -5-C-(3-hydroxybutyl)hyacinthacine A₂, Hyacinthacine B₇, C₂, C₃, C₄ and C₅ were isolated and tested against a panel of selected glycosidases. Of these results, hyacinthacine C₂, C₃ and C₅ proved noteworthy activity against *C. saccharolyticum* β -glucosidase with IC₅₀ values of 13 μ M, 25 μ M and 48 μ M, respectively. Hyacinthacine C₄ proved to be the only active compound against rice α -glucosidase (IC₅₀ = 110 μ M), but shared similar activities with hyacinthacine C₅ against rat α -glucosidase (IC₅₀ = 45 μ M and 77 μ M, respectively) and the amyloglucosidase of *A. niger* (IC₅₀ = 57 μ M for both C₄ and C₅). Hyacinthacine C₃ displayed a unique inhibition of bovine liver β -galactosidase with an IC₅₀ value of 52 μ M, whilst hyacinthacine C₂ proved the only alkaloid capable of inhibiting human placenta α -L-fucosidase with an IC₅₀ value of 17 μ M [50].

2.2. Current Issues with the Hyacinthacine Alkaloids

Excluding the three long-chain hyacinthacine A-type analogues, 19 hyacinthacine type alkaloids have been isolated and reported between 1999 and 2007. In most cases, 2D NMR experiments (gCOSY, HSQC, HMBC) were the sole basis of structure elucidation. Moreover, NOE correlation as well as ³J_{H,H} coupling

Table 1. Concentration (μM) of the naturally isolated hyacinthacine alkaloids giving 50% inhibition of various glycosidases (IC_{50} values).

		IC_{50} (μM)			
					
enzyme		Hyacinthacine A ₁	Hyacinthacine A ₂	Hyacinthacine A ₃	Hyacinthacine A ₄
α-glucosidase					
	Yeast	- ^a	-	-	NI
	Rice	240	NI	NI	NI
	Rat intestinal maltase	NI ^b	NI	NI	NI
	Rat liver lysosome	-	-	-	NI
β-glucosidase					
	Almond	250	150	NI	-
	<i>Caldocellum saccharolyticum</i>	-	-	-	NI
	Human Placenta	-	-	-	-
α-galactosidase					
	Coffee beans	-	-	-	-
β-galactosidase					
	Bovine liver	-	-	-	NI
	Rat intestinal lactase	4.4	73	160	-
	Rat epididymis	-	-	-	-
α-mannosidase					
	Jack bean	NI	NI	NI	-
	Rat epididymis	-	-	-	-
β-mannosidase					
	Snail	-	-	-	-
	Rat epididymis	390	1000	NI	NI
α-L-fucosidase					
	Bovine epididymis	-	-	-	NI
	Rat epididymis	46	NI	NI	-
	Human placenta	-	-	-	-
α,α-trehalase					
	Porcine kidney	NI	260	NI	-
amyloglucosidase					
	<i>Aspergillus niger</i>	25	8.6	17	NI
α-L-rhamnosidase					
	<i>Penicillium decumbens</i>	-	-	-	NI

^a“-” : Not tested, ^bNI : No inhibition (less than 50 % inhibition at 1000 μM)

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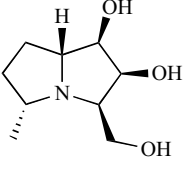
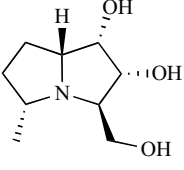
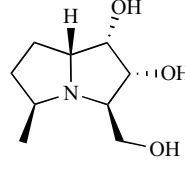
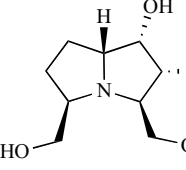
	IC ₅₀ (μM)			
				
Enzyme	Hyacinthacine A ₅	Hyacinthacine A ₆	Hyacinthacine A ₇	Hyacinthacine B ₁
α-glucosidase				
Yeast	NI	NI	NI	-
Rice	NI	NI	NI	NI
Rat intestinal maltase	NI	NI	NI	NI
Rat liver lysosome	NI	NI	NI	-
β-glucosidase				
Almond	-	-	-	320
<i>Caldocellum saccharolyticum</i>	NI	NI	NI	520
Human placenta	-	-	-	-
α-galactosidase				
Coffee beans	-	-	-	-
β-galactosidase				
Bovine liver	NI	NI	NI	110
Rat intestinal lactase	-	-	-	270
Rat epididymis	-	-	-	-
α-mannosidase				
Jack bean	-	-	-	-
Rat epididymis	-	-	-	-
β-mannosidase				
Snail	-	-	-	NI
Rat epididymis	NI	NI	NI	-
α-L-fucosidase				
Bovine epididymis	NI	NI	NI	NI
Rat epididymis	-	-	-	-
Human placenta	-	-	-	-
α,α-trehalase				
Porcine kidney	-	-	-	NI
amyloglucosidase				
<i>Aspergillus niger</i>	110	NI	NI	NI
α-L-rhamnosidase				
<i>Penicillium decumbens</i>	NI	NI	NI	-

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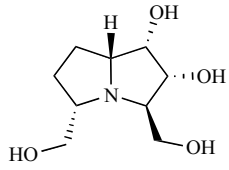
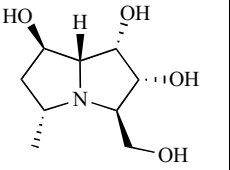
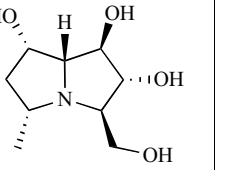
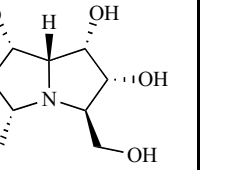
	IC ₅₀ (μM)			
				
Enzyme	Hyacinthacine B ₂	Hyacinthacine B ₃	Hyacinthacine B ₄	Hyacinthacine B ₅
α-glucosidase				
Yeast	-	-	NI	NI
Rice	NI	NI	NI	NI
Rat intestinal maltase	NI	NI	NI	NI
Rat liver lysosome	-	-	NI	NI
β-glucosidase				
Almond	100	NI	-	-
<i>Caldocellum saccharolyticum</i>	490	-	NI	NI
Human placenta	-	-	-	-
α-galactosidase				
Coffee beans	-	-	-	-
β-galactosidase				
Bovine liver	160	-	NI	NI
Rat intestinal lactase	3.6	18	-	-
Rat epididymis	-	-	-	-
α-mannosidase				
Jack bean	-	NI	-	-
Rat epididymis	-	-	-	-
β-mannosidase				
Snail	NI	-	-	NI
Rat epididymis	-	NI	NI	-
α-L-fucosidase				
Bovine epididymis	NI	-	23	NI
Rat epididymis	-	850	-	-
Human placenta	-	-	-	-
α,α-trehalase				
Porcine kidney	NI	NI	-	-
amyloglucosidase				
<i>Aspergillus niger</i>	NI	51	89	110
α-L-rhamnosidase				
<i>Penicillium decumbens</i>	-	-	NI	NI

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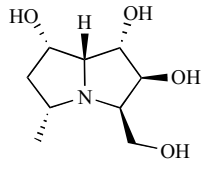
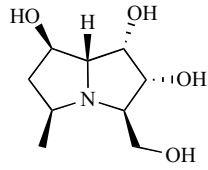
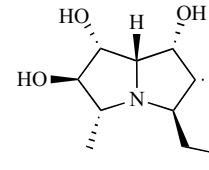
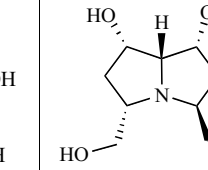
	IC ₅₀ (μM)			
				
Enzyme	Hyacinthacine B ₆	Hyacinthacine B ₇	Hyacinthacine C ₁ (1999)	Hyacinthacine C ₂
α-glucosidase				
Yeast	NI	-	-	-
Rice	NI	NI	NI	NI
Rat intestinal maltase	NI	NI	NI	NI
Rat liver lysosome	NI	-	-	-
β-glucosidase				
Almond	-	-	NI	-
<i>Caldocellum saccharolyticum</i>	NI	NI	NI	13
Human placenta	-	-	-	-
α-galactosidase				
Coffee beans	-	NI	-	NI
β-galactosidase				
Bovine liver	NI	NI	NI	370
Rat intestinal lactase	-	-	NI	-
Rat epididymis	-	-	-	-
α-mannosidase				
Jack bean	-	NI	-	NI
Rat epididymis	-	-	-	-
β-mannosidase				
Snail	-	-	NI	-
Rat epididymis	NI	-	-	-
α-L-fucosidase				
Bovine epididymis	NI	-	NI	-
Rat epididymis	-	-	-	-
Human Placenta	-	NI	-	17
α,α-trehalase				
Porcine kidney	-	-	NI	-
amyloglucosidase				
<i>Aspergillus niger</i>	NI	270	84	550
α-L-rhamnosidase				
<i>Penicillium decumbens</i>	NI	-	-	-

Table 1. contd...

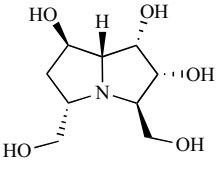
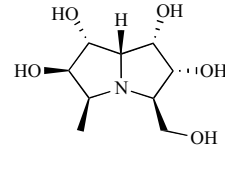
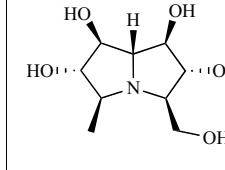
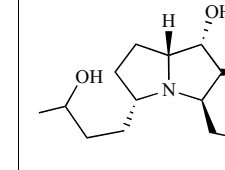
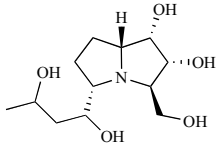
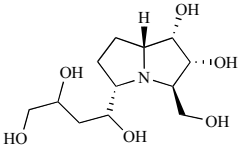
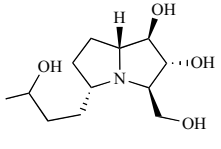
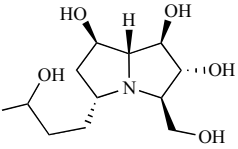
	IC ₅₀ (μM)			
				
Enzyme	Hyacinthacine C ₃	Hyacinthacine C ₄ (2007)	Hyacinthacine C ₅	α-5-C-(3-hydroxybutyl)hyacinthacine A ₁
α-glucosidase				
Yeast	-	-	-	NI
Rice	NI	110	NI	NI
Rat intestinal maltase	NI	45	77	NI
Rat liver lysosome	-	-	-	-
β-glucosidase				
Almond	-	-	-	49
<i>Caldocellum saccharolyticum</i>	25	660	48	136
Human placenta	-	-	-	NI
α-galactosidase				
Coffee beans	NI	NI	NI	NI
β-galactosidase				
Bovine liver	52	890	900	NI
Rat intestinal lactase	-	-	-	-
Rat epididymis	-	-	-	NI
α-mannosidase				
Jack bean	NI	NI	NI	-
Rat epididymis	-	-	-	NI
β-mannosidase				
Snail	-	-	-	-
Rat epididymis	-	-	-	NI
α-L-fucosidase				
Bovine epididymis	-	-	-	NI
Rat epididymis	-	-	-	-
Human Placenta	NI	NI	NI	-
α,α-trehalase				
Porcine kidney	-	-	-	-
amyloglucosidase				
<i>Aspergillus niger</i>	870	57	57	-
α-L-rhamnosidase				
<i>Penicillium decumbens</i>	-	-	-	NI

Table 1. contd...

	IC ₅₀ (μM)			
				
Enzyme	α-5-C-(1,3-dihydroxybutyl)hyacinthacin e A ₁	α-5-C-(1,3,4-trihydroxybutyl)hyacinthacin A ₁	α-5-C-(3-hydroxybutyl)hyacinthacin A ₂	α-5-C-(3-hydroxybutyl)-7-epi-australine
α-glucosidase				
Yeast	3.6	650	-	6.6
Rice	300	390	NI	NI
Rat intestinal maltase	350	NI	NI	NI
Rat liver lysosome	-	-	-	-
β-glucosidase				
Almond	9.5	25.4	-	NI
<i>Caldocellum saccharolyticum</i>	5.1	11.4	NI	200
Human placenta	NI	NI	-	NI
α-galactosidase				
Coffee beans	NI	NI	NI	NI
β-galactosidase				
Bovine liver	830	920	NI	320
Rat intestinal lactase	-	-	-	-
Rat epididymis	NI	NI	-	NI
α-mannosidase				
Jack bean	-	-	NI	-
Rat epididymis	680	NI	-	NI
β-mannosidase				
Snail	-	-	-	-
Rat epididymis	NI	NI	-	NI
α-L-fucosidase				
Bovine epididymis	NI	NI	-	NI
Rat epididymis	-	-	-	-
Human Placenta	-	-	NI	-
α,α-trehalase				
Porcine kidney	-	-	-	-
amyloglucosidase				
<i>Aspergillus niger</i>	-	-	180	-
α-L-rhamnosidase				
<i>Penicillium decumbens</i>	180	NI	-	NI

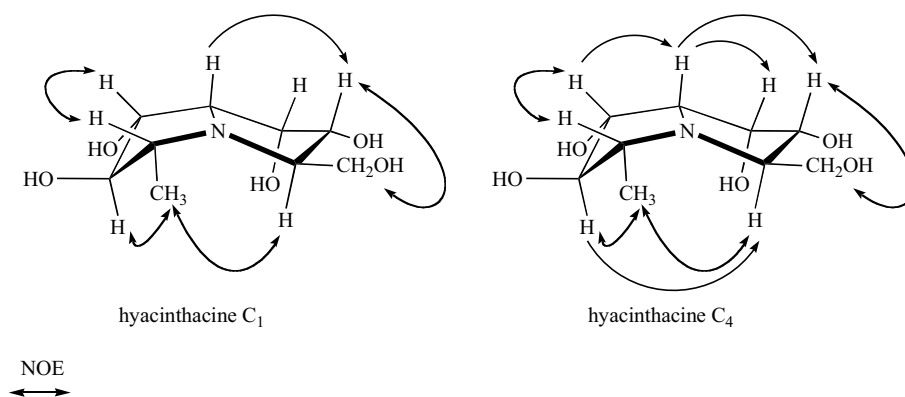


Fig. (7). The NOE correlations reported for hyacinthacine C₁ [41] and C₄ [50].

patterns and constants were used to determine the relative configuration but were limited such that they were unable to identify the absolute configuration of the isolated hyacinthacines. With no X-ray crystallographic structures of these complex natural isolates, this has understandably led to inconsistencies among the hyacinthacines and suggests that their assigned configurations are not secure. An immediately obvious example of this can be seen in the proposed structures of hyacinthacines C₁ and C₄. Although reported nearly eight years apart, both alkaloids were assigned with identical structures despite their prominent differences in both ¹H and ¹³C NMR spectroscopic data and their optical rotations. Despite plausible NOE correlations presented for both structures (Fig. 7), the aforementioned differences between the spectroscopic data suggest at least one of these structures be incorrect [41, 50].

Therefore, to confirm their structures, including the proposed relative and absolute configurations, the hyacinthacine alkaloids have been of recent interest for total synthesis. Furthermore, total synthesis also allows access to the core structure of these complex alkaloids and so presents a great significance to the large community interested in the preparation of glycosidase inhibitors and consequent therapeutic iminosugars.

To date, fourteen of the natural hyacinthacines, A₁, A₂, A₃, A₆, A₇, B₁, B₂, B₃, B₄, B₅, B₇, C₂, C₃ and C₅ have been synthesized, along with their related epimers and enantiomers [51-64]. At this point, the authors note that an increasing number of reports suggest the confirmation of hyacinthacine A₅ through methods of total synthesis [61, 63, 65]. To the best of our knowledge, hyacinthacine A₅ has not yet been synthesized and although epimers of the structure have been reported [66], the absolute configuration of the natural isolate is still yet to be confirmed. In addition to the aforementioned issues, recent synthetic studies have also revealed that the original structures proposed for hyacinthacine B₇, C₃ and C₅ are incorrect [58-62]. It, therefore, becomes apparent that structural revision of the original isolates is necessary because as demonstrated in the earlier table, hyacinthacine alkaloid epimers determine the degree of specificity as well as the potency for the inhibition of various glycosidases.

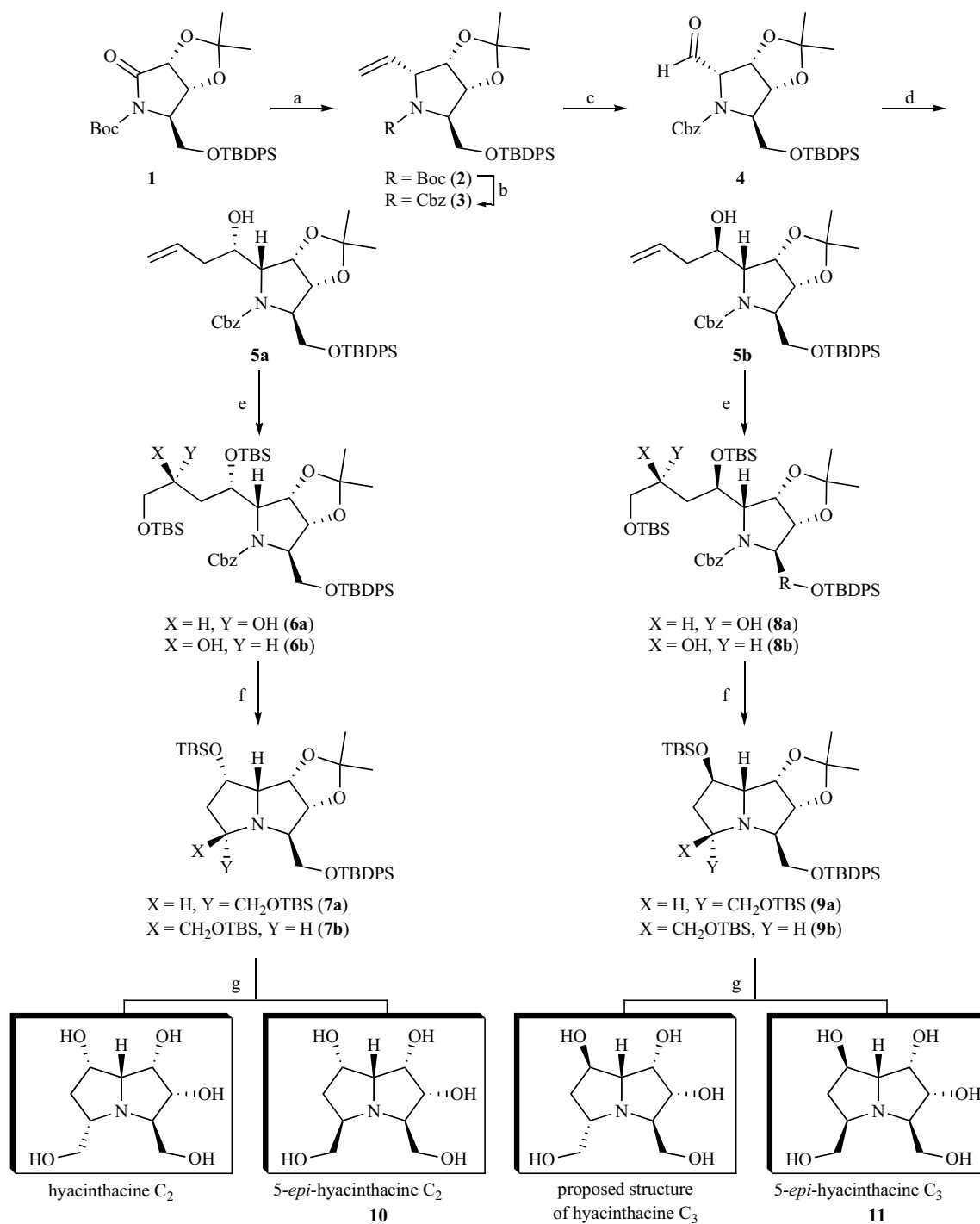
2.3. Total Syntheses of the Hyacinthacine Alkaloids

Each subclass of the hyacinthacine alkaloids displays relatively low glycosidase inhibitory activities and so merits their total synthesis. Relative to A-type hyacinthacines, both B and C-type alkaloids have greater constitutional complexity and as a result, have led to a greater number of insecure structural assignments. Synthetic work towards hyacinthacine A and B-type alkaloids has been included in a number of reviews [67-69], with a comprehensive analysis completed in 2012 by Pyne *et al.* [70]. The hyacinthacine C-type alkaloids on the other hand have attracted considerable interest and there has been a number of publications

towards their synthetic work that has not yet been summarised. When considered from a structural point of view, the hyacinthacine C-type alkaloids are the most diverse subclass of the hyacinthacines. They contain at least six possible stereogenic centres, which means that there is a minimum of 32 unique possible diastereomers (plus their enantiomers) that can contain a 3-hydroxymethyl-5-methylpyrrolizidine-1,2,6,7-tetraol core. Their high degree of oxidation presents a remarkably analogous structure to glucose and is, therefore, an enticing, but challenging synthesis for chemists interested in accessing medicinally useful iminosugars. For these reasons, the remainder of this report will largely focus on the hyacinthacine C-type alkaloids and will aim to deliver a comprehensive and collective review of all the different synthetic approaches towards this subclass of hyacinthacine.

2.4. Yoda *et al.* 2009

The first synthesis of hyacinthacine C-type alkaloids was the synthesis of hyacinthacine C₂ and C₃ along with their C5-epimers reported by Yoda *et al.* in 2009 [60]. This synthesis was an initial attempt to reveal information regarding structure-activity relationships between the hyacinthacine C-type alkaloids and their inhibition against various glycosidases. Their synthetic strategy (Scheme 1) starts with the preparation of the *N*-Boc lactam **1** from commercially available (*S*)-(-)-2-pyrrolidone-5-carboxylic acid. The *N*-Boc lactam **1** was subjected to a Grignard addition followed by a 1,2-reduction of the resulting unsaturated ketone formed by pyrrolidinone ring opening to give the corresponding allyl alcohol. Subsequent mesylation-cyclization then afforded the *N*-Boc-**2** product. Next, the Boc protecting group was replaced with Cbz in a high yielding, three steps, procedure to give *N*-Cbz-**3**. The olefin moiety of *N*-Cbz-**3** was then subjected to oxidative cleavage with OsO₄ and NaIO₄ to give aldehyde **4** as a single isomer. A Reformatsky-type allylation of **4** returned a 79:21 separable mixture of the alcohols **5a** and **5b**, respectively. To establish the methodology, alcohol **5a** then underwent TBS protection of the hydroxy group followed by a non-selective dihydroxylation of the terminal olefin moiety. The inseparable diol mixture was then subjected to a selective TBS protection of the primary alcohol, affording a high yield (92%) of a 1:1 mixture of separable diastereomers **6a** and **6b**. In separate reactions, mesylation of the secondary alcohols of **6a** and **6b** followed by Cbz deprotection resulted in concerted cyclization to give the pyrrolizidines **7a** and **7b**. Individual global deprotection of both pyrrolizidines afforded hyacinthacine C₂ along with its epimer, 5-*epi*-hyacinthacine C₂ **10** in 72% and 71% yields, respectively. Having established the synthetic pathway to hyacinthacine C₂, Yoda and co-workers employed a similar synthetic methodology to alcohol **5b** where they also successfully synthesized hyacinthacine C₃ along with its epimer, 5-*epi*-hyacinthacine C₃ **11**. When compared with the spectroscopic data of the natural isolates [50], Yoda *et al.*

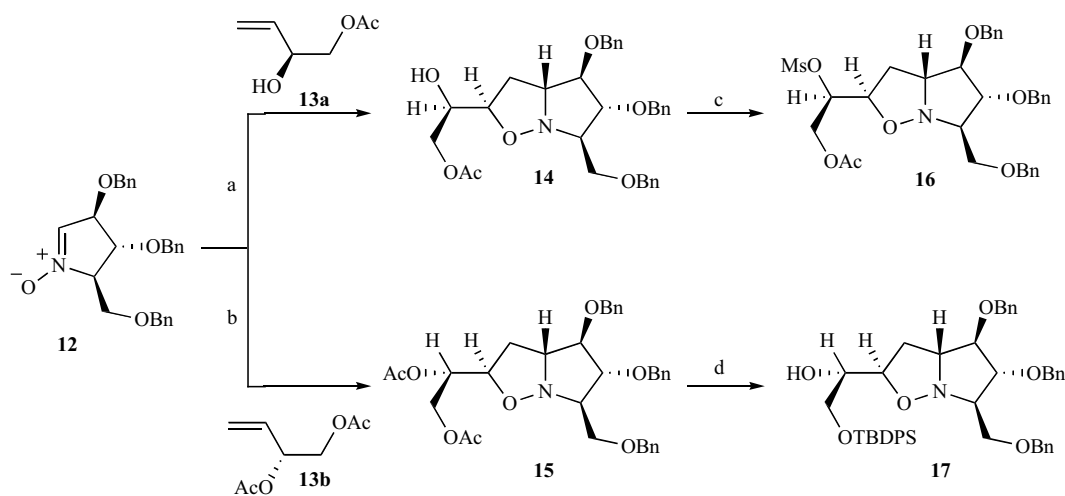


Scheme 1. The first total synthesis of hyacinthacine C₂ and proposed structure of C₃ and their C5 epimers **10** and **11**, respectively by Yoda *et al.* [60].

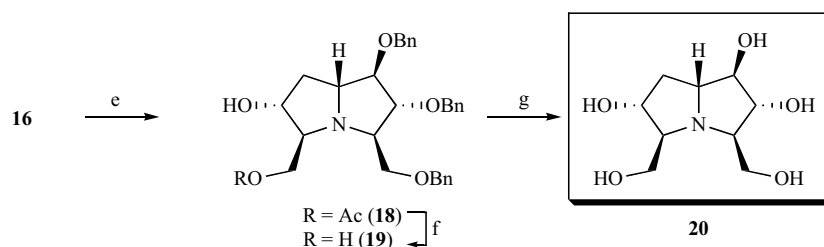
confirmed only the structure for natural hyacinthacine C₂ was correct. They found that the characterization data of their synthetic sample of hyacinthacine C₃ was inconsistent with the corresponding natural product.

2.5. Tamayo *et al.* 2009

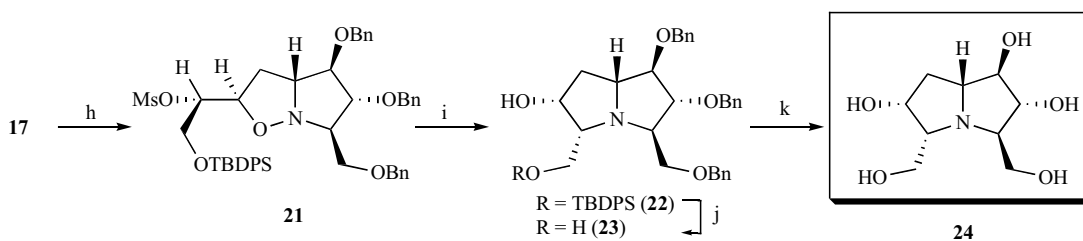
At around the same time as Yoda *et al.*, Tamayo and co-workers published their work detailing the synthetic approach to making analogues of hyacinthacines C_{2/3} from the arabinose



Reagents and conditions: (a) **13a**, CH₂Cl₂, mW, 70°C, 2.5 h, 70%; (b) **13b**, CH₂Cl₂, MW, 70°C, 4 h, 74%; (c) MsCl, pyridine, CH₂Cl₂, 74%; (d) (i) Ambersep® 900 (OH) MeOH, quant.; (ii) TBDPSCl, imidazole, DMF, 92%.



Reagents and conditions: (e) Zn, AcOH/H₂O (9:1), 60°C, 60%; (f) Ambersep® 900 (OH) MeOH, 83%; (g) (i) H₂, 10% Pd/C, HCl, MeOH; (ii) Dowex 50WX8 NH₄OH, 85%.



Reagents and conditions: (h) MsCl, Pyridine, CH₂Cl₂, 81%; (i) Zn, AcOH/H₂O (9:1), 60°C, 76%; (j) TBAF, THF, 61% (k) (i) H₂, 10% Pd/C, HCl, MeOH; (ii) Dowex 50WX8 NH₄OH, 90%.

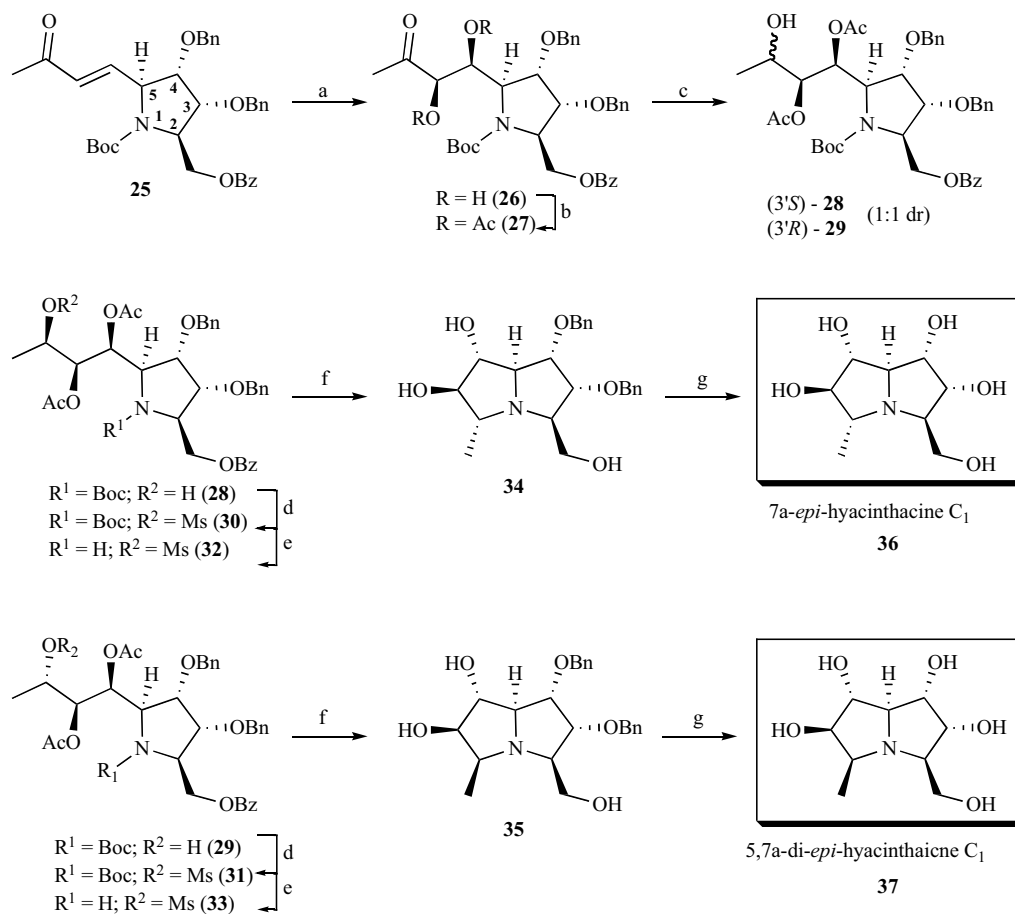
Scheme 2. The total synthesis of unnatural hyacinthacine C₂₃ analogues **20** and **24** by Tamayo *et al.* [71].

derived nitrone **12** [71] (Scheme 2). Their synthesis began with the 1,3-dipolar cycloaddition of the chemoenzymatically prepared 3-buten-1,2-diol derivatives **13a** or **13b** to nitrone **12** to afford cycloadducts **14** or **15**, respectively. At this stage, methodological probing was performed with the pyrroloisoxazolidine **14**, which was subsequently converted into the mesylate **16**. Compound **16** underwent an N-O reduction with Zn/AcOH, which after a basic workup, spontaneously cyclized to give the pyrrolizidine **18**. Deacetylation to give **19**, followed by catalytic hydrogenation under acidic conditions afforded the final product **20**. After access to the pyrrolizidine core was established, Tamayo and co-workers focused their attention on cycloadduct **15**. More specifically, deacetylation of **15**, followed by a TBDPS protection of the primary alcohol afforded **17** in nearly quantitative yields. Using similar methodology (mesylation, N-O reduction, global deprotection), the 5-epimer **24** was obtained. Although they were not assessed as potential glycosidase inhibitors, access to these highly oxygenated

iminosugars is still important for the broader community interested in synthesizing complex aza-sugars.

2.6. Tamayo *et al.* 2010

At this stage, hyacinthacines C₁, C₄ and C₃ proved to be insecure in their structural assignment. Their glycosidase inhibitory activities proved noteworthy, but difficult to predict, especially due to these inconsistencies. For these reasons, Tamayo and co-workers were led to investigate and report the synthesis of unnatural 7*a*-*epi*-hyacinthacine C₁ **36** and 5,7*a*-*di-epi*-hyacinthacine C₁ **37** in 2010 [72]. From their previous work towards the synthesis of hyacinthacine A-type alkaloids [73], Tamayo *et al.* recognized that using the common precursor, the α,β -unsaturated ketone **25**, would afford access to both hyacinthacine C-type alkaloids (Scheme 3). More specifically, **25** was subjected to a catalytic dihydroxylation using OsO₄ and NMO to afford a single diol diastereomer **26** in



Reagents and conditions: (a) OsO_4 , NMO, acetone/ H_2O (8:1), 71%; (b) Ac_2O , pyridine, 86%; (c) NaBH_4 , MeOH, 0°C , 25% (**28**), 22% (**29**); (d) MsCl , Et_3N , CH_2Cl_2 , 75% (**30**), 83% (**31**); (e) TFA, CH_2Cl_2 , 83% (**32**), 90% (**33**); (f) Et_3N , THF reflux, then NaOMe , 60% (**34**), 67% (**35**); (g) H_2 , 10% Pd/C, HCl, MeOH, 68% (7*a*-*epi*-hyacinthacine C_1 (**36**), 70% (5,7*a*-*di-epi*-hyacinthacine C_1 (**37**)).

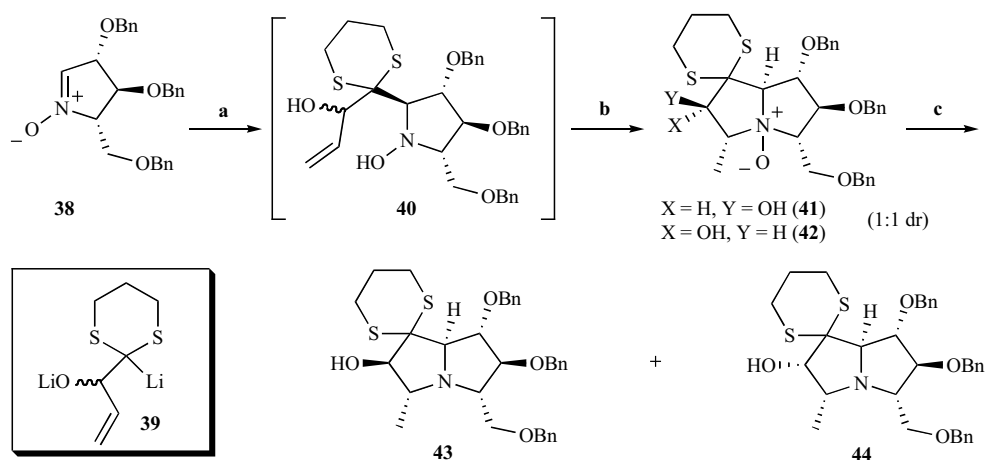
Scheme 3. The synthesis of unnatural hyacinthacine C_1 analogues, 7*a*-*epi*-hyacinthacine C_1 **36** and 5,7*a*-*di-epi*-hyacinthacine C_1 **37** by Tamayo *et al.* [72].

high yield. The high diastereoselectivity achieved is a result of the steric hindrance created by both the Boc protecting group at the amino moiety as well as the benzyl groups at C-4 and C-3. Diol **26** was then acetylated in good yield to afford the di-*O*-acetylated **27**, which was subsequently reduced with NaBH_4 to afford a separable mixture of alcohols **28** and **29** in a 1:1 ratio (isolated yields of 25% and 22%, respectively). To prepare the pyrrolizidine core, alcohols **28** and **29** underwent separate mesylation reactions, followed by an acid-catalysed *N*-Boc deprotection to give the secondary amines **32** and **33**, respectively. After heating at reflux in THF under basic conditions, both amines cyclised to give their respective pyrrolizidine cores. To both pyrrolizidine mixtures were then added MeONa in MeOH to facilitate a one-pot debenzoyl- and deacetylation to afford triols **34** and **35**, respectively. Final *O*-benzyl deprotection of **34** and **35** revealed the desired hyacinthacine C-type analogues, 7*a*-*epi*-hyacinthacine C_1 **36** and 5,7*a*-*di-epi*-hyacinthacine C_1 **37**, respectively. Although neither was able to resolve the structural discrepancies in the literature, access to stereogenic variations of the heavily hydroxylated pyrrolizidine core proves important. Their therapeutic potential remains unclear as these compounds were not assessed for their glycosidase inhibition.

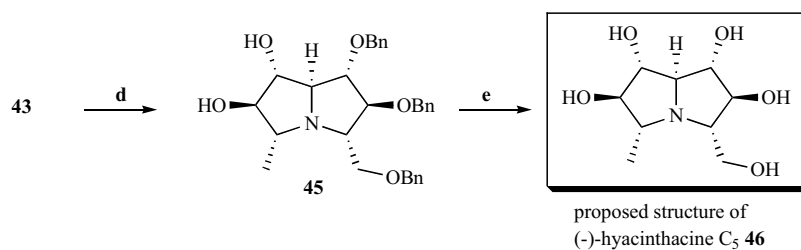
2.7. Yu *et al.* 2011

In the following year, Yu *et al.* published their findings regarding the synthesis of (–)-hyacinthacine C_5 , 6-*epi*-(–)-

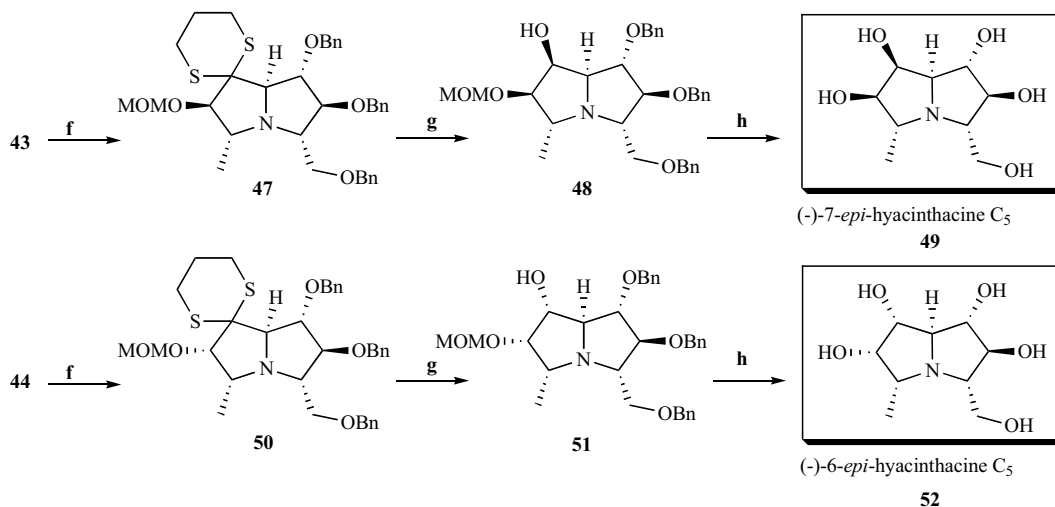
hyacinthacine C_5 and 7-*epi*-(–)-hyacinthacine C_5 from the L-arabinose derived cyclic nitron **38** [61] (Scheme 4). This not only expanded the number of hyacinthacine C-type analogues synthesized, but it was also the first synthesis of the enantiomer of natural isolate hyacinthacine C_5 . Their synthesis began with the addition of the lithiated dithiane **39** to the cyclic nitron **38** which returned the stereoselective hydroxylamine intermediate **40**. Compound **40** was then subjected to Cope-House cyclization conditions which afforded the separable pyrrolizidine *N*-oxides **41** and **42** in a 1:1 ratio (55% combined yield). Pyrrolizidine *N*-oxides **41** and **42** were then separately reduced using Zn-HOAc to afford the pyrrolizidines **43** and **44**, respectively. Initially focusing on the enantiomer of hyacinthacine C_5 , the dithioketal moiety of pyrrolizidine **43** was hydrolysed to its corresponding ketone, which was then selectively reduced to the corresponding diol **45** in an overall 31% yield. Subsequent debenzoylation revealed the final product as (–)-hyacinthacine C_5 . Turning their attention to the related epimers, Yu and colleagues initially protected the free hydroxy moiety in **43** using MOMCl, followed by hydrolysis of the dithioketal to give the corresponding ketone in 61% yield. A stereoselective reduction of the ketone with NaBH_4 afforded two separable diastereoisomers favouring the desired alcohol **48** in a 5:1 ratio with an overall high yield (95.5%). Global deprotection of the MOM and the *O*-benzyl groups afforded (–)-7-*epi*-hyacinthacine C_5 **49**. Having established the synthetic pathway, Yu and co-workers successfully synthesized (–)-6-*epi*-hyacinthacine C_5 **52** by



Reagents and conditions: (a) TMEDA, **39**, THF, -30 °C; (b) CHCl₃, 28% (**41**), 27% (**42**) (two steps); (c) Zn, HOAc, 98% (**43**), 98%, (**44**).



Reagents and conditions: (d) (i) NBS, AgNO₃, MeCN/H₂O (4:1); (ii) NaBH₄, MeOH, 31% (two steps); (e) H₂, 10% Pd/C, HCl, MeOH, 93%.



Reagents and conditions: (f) NaH, MOMCl, THF, 76% (**47**), 71% (**50**); (g) (i) NBS, AgNO₃, MeCN/H₂O (4:1); (ii) NaBH₄, MeOH; (iii) HCl, MeOH, reflux; (h) H₂, 10% Pd/C, HCl, MeOH, 41% (7-*epi*-(-)-hyacinthacine C₅ (**49**) over four steps), 50% (6-*epi*-(-)-hyacinthacine C₅ (**52**) over four steps).

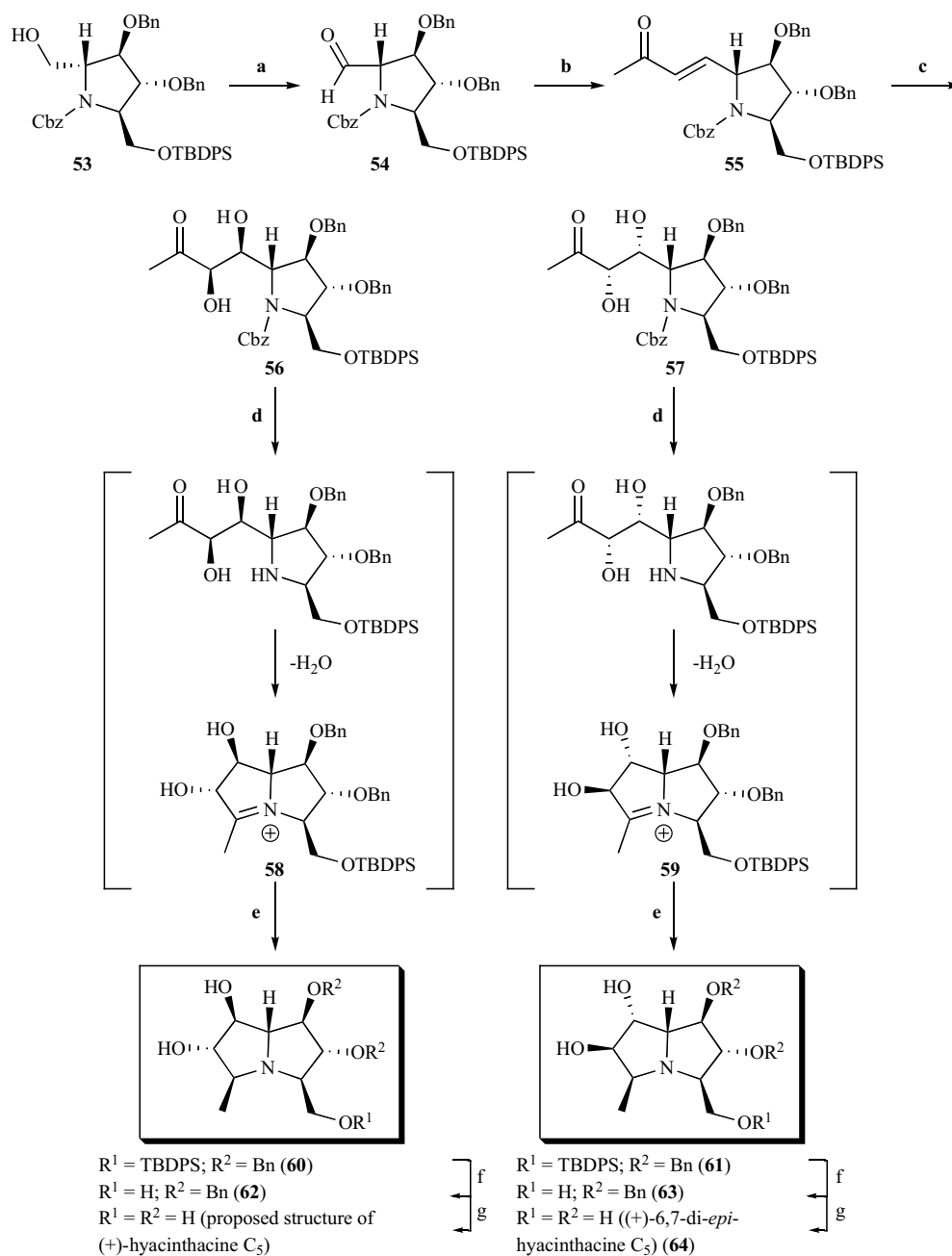
Scheme 4. A total synthesis of the proposed structure of (-)-hyacinthacine C₅, (-)-6-*epi*-hyacinthacine C₅ **52** and (-)-7-*epi*-hyacinthacine C₅ **49** by Yu *et al.* [61].

subjecting **44** to the similar methodology. With the three products in hand, Yu *et al.* reported that none of the ¹H NMR and ¹³C NMR spectroscopic data matched with those reported for the natural isolate labelled (+)-hyacinthacine C₅ [50]. This led to the conclusion that natural (+)-hyacinthacine C₅ was in fact, a different isomer. Regardless, all synthetic isomers of this study were assayed as potential glycosidase inhibitors and found that (-)-6-*epi*-

hyacinthacine C₅ **52** is a weak inhibitor against the α-glucosidases of rat intestinal maltase (IC₅₀ = 58.5 μM) and rice (IC₅₀ = 64.2 μM).

2.8. Tamayo *et al.* 2011

At the same time as Yu *et al.* and for similar reasons, Tamayo and co-workers also published related work detailing a synthetic approach towards (+)-hyacinthacine C₅ along with its C₆, C₇-

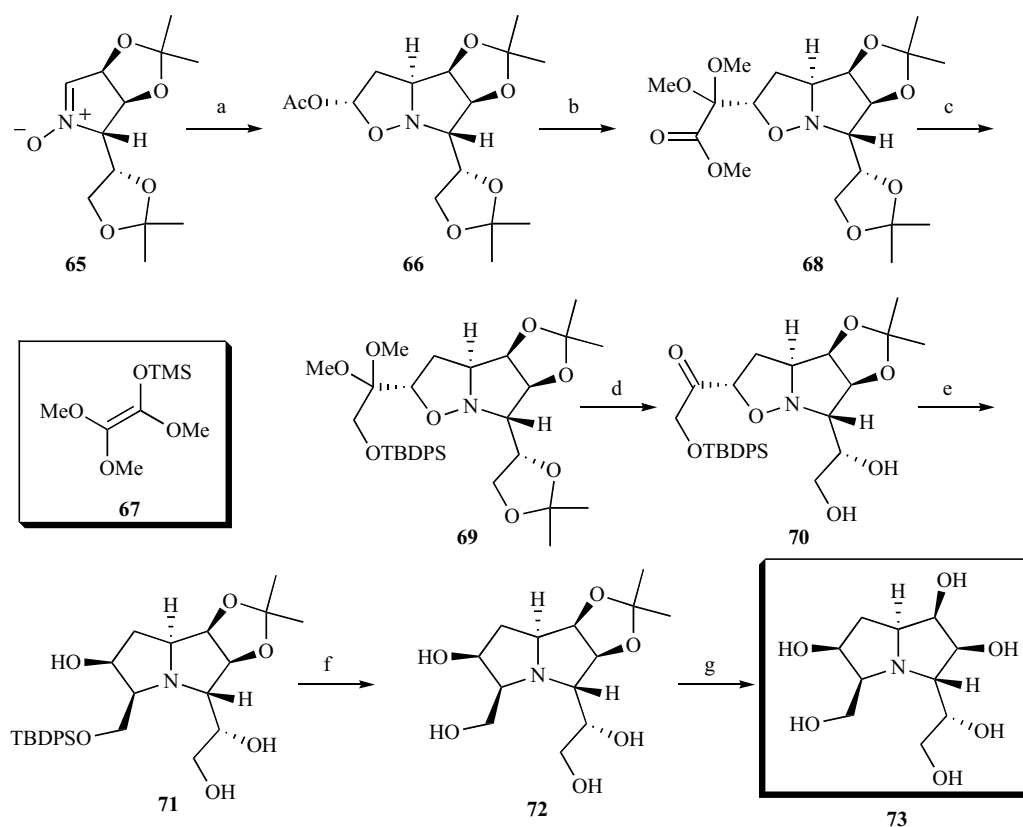


Reagents and conditions: (a) TPAP, CH_2Cl_2 , 4 Å MS, 95%; (b) $\text{Ph}_3\text{PCHCOMe}$, PhMe, reflux, 91%; (c) OsO_4 , NMO, acetone/ H_2O (8:1), 37% (**56**), 28% (**57**); (d-e) H_2 , 10% Pd/C, MeOH, 65% (**60**), 56% (**61**); (f) TBAF, THF, 94% (**62**), 76% (**63**); (g) H_2 , 10% Pd/C, MeOH, 73% ((+)-hyacinthacine C_5), 99% ((+)-6,7-di-*epi*-hyacinthacine C_5) (**64**).

Scheme 5. The total synthesis of the proposed structure of (+)-hyacinthacine C_5 and (+)-6,7-di-*epi*-hyacinthacine C_5 by Tamayo *et al.* [62].

diepimer [62]. Their synthesis began with the *N*-Cbz protected pyrrolidine **53**, a 2,5-dideoxy-2,5-imino-D-mannitol (DMDP) derivative (Scheme 5). In this synthesis, the primary alcohol of **53** was initially oxidised its corresponding aldehyde **54**, which was further treated *in situ* with 1-triphenylphosphoranylidene-2-propanone to afford the α,β -unsaturated ketone **55**. A non-selective dihydroxylation of **55** with OsO_4 and NMO returned a 1.4:1 separable mixture of diols **56** and **57**, respectively. Individually, both diols **56** and **57** were subjected to reductive amination to form their respective iminium intermediates **58** and **59**. Prolonged exposure to catalytic hydrogenation conditions over Pd/C resulted in stereospecific hydrogenation occurring *anti* to the sterically

encumbering CH_2OTBDPS group of both pyrrolizidines. More specifically, both pyrrolizidines **60** and **61** were synthesized with the same C_5 -stereochemistry. Individual global deprotections of pyrrolizidines **60** and **61**, which included a desilylation and hydrogenolysis of the benzyl protecting groups, revealed both (+)-hyacinthacine C_5 and (+)-6,7-di-*epi*-hyacinthacine C_5 **64**, respectively. Arriving at a similar conclusion made by Yu *et al.*, Tamayo and colleagues also found that neither of their spectroscopic data for hyacinthacine C_5 and its C_6 , C_7 -diepimer **64** matched that of the natural isolate [50] further suggesting it to be a different isomer. The final products of this study were not assessed for their glycosidase inhibitory activities.



Reagents and conditions: (a) vinyl acetate, sealed tube, 75 °C, 78%; (b) TMSOTf, **67**, CH₂Cl₂, -80 °C, 66%; (c) (i) LiAlH₄, THF, 0 °C, 89%; (ii) TBDPSCl, imidazole, CH₂Cl₂, 94%; (d) AcOH/H₂O (4:1), 60 °C, 55%; (e) H₂, 10% Pd/C, EtOH, 79%; (f) TBAF, THF, 80%; (g) TFA/H₂O (1:1), 95%.

Scheme 6. A total synthesis of an unnatural hyacinthacine C₂₃ analogue **73** by Fischer *et al.* [74].

2.9. Fischer *et al.* 2014

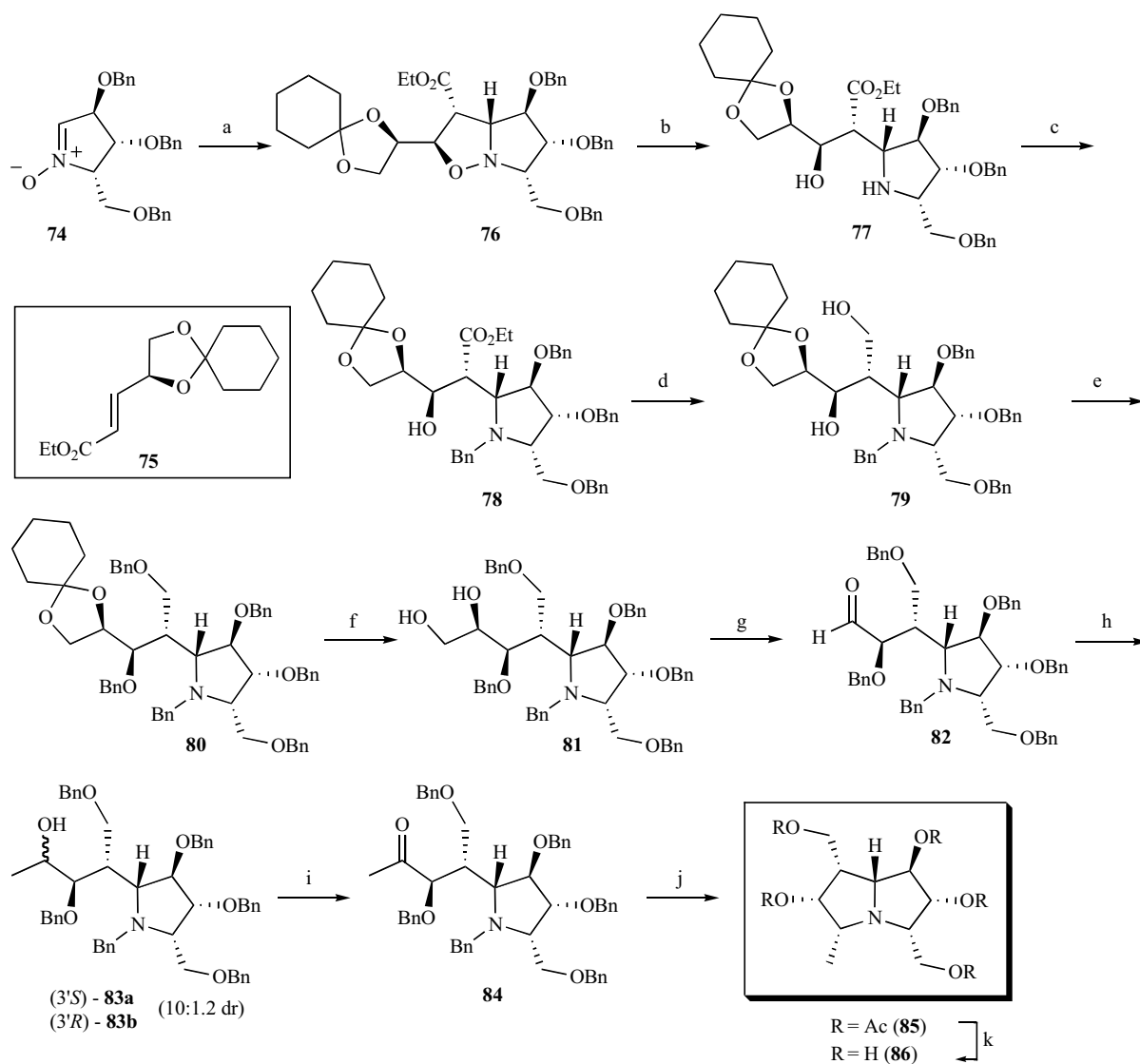
By 2014, synthetic work towards the hyacinthacine C-type alkaloids had revealed several structural inconsistencies among their natural isolates. For this reason, and to also expand the scope of information pertaining to these compounds, Fischer and co-workers investigated the usefulness of asymmetric 1,3-dipolar cycloadditions to prepare optically active nitron templates towards making various polyhydroxylated pyrrolizidine analogues [74]. One of the iminosugars prepared by Fischer and co-workers can be considered an unnatural hyacinthacine C-type alkaloid shown in Scheme 6. To obtain this analogue, Fischer *et al.* began their synthesis through the preparation of the mannose-derived nitron **65** (Scheme 6). The cyclic nitron **65** underwent a 1,3-dipolar cycloaddition with vinyl acetate and afforded a 9:1 isolatable mixture of isoxazolidines, favouring the formation of **66** for steric reasons. The acetate moiety of **66** then underwent an S_N1-like reaction with silylketene acetal **67**, which exclusively produced the 2,3a-*trans*-isoxazolidine **68** in a 69% yield. In a two-step procedure, the methyl ester of isoxazolidine **68** was initially reduced with LiAlH₄, then subsequently protected with *tert*-butyl(diphenyl)silyl chloride to give isoxazolidine **69** in good yield (84% over two steps). The dimethoxy ketal moiety in compound **69** was then hydrolysed to the ketone to give product **70**, which was subjected to N-O bond reduction and then reductive amination by hydrogenation over Pd/C to afford the pyrrolizidine **71** as a single isomer. A global deprotection including the removal of the silyl and the isopropylidene moieties returned the hexahydroxylated pyrrolizidine **73** in 76% yield over two steps.

2.10. Vankar *et al.* 2014

In the same year, Vankar and co-workers reported their synthetic work towards the synthesis of homoanalogues of hyacinthacine C₅ [75]. Their 2014 report details starting from the arabinose-based cyclic nitron **74** which underwent a 1,3-dipolar cycloaddition reaction with the D-mannitol-aldehyde derived dipolarophile **75** (Scheme 7). The desired cycloadduct **76** was obtained in a 9:1 ratio in a combined yield of 88%. Compound **76** was then subjected to a Zn/HOAc mediated N-O cleavage which produced the pyrrolidine **77** which then underwent selective benzylation at the secondary amine to give **78** in 85% over two steps. The ester moiety of **78** was then reduced to its corresponding alcohol to afford diol **79**. A non-selective *O*-benzylation of both hydroxy groups afforded **80**, which upon removal of the cyclohexylidene acetal, resulted in the formation of the diol **81**. The diol moiety of compound **81** was then oxidatively cleaved with sodium periodate to give aldehyde **82** which was then treated with MeMgI, returning a selective 10:1.2 mixture of diastereomers **83a** and **83b**. The diastereomeric mixture was then oxidised to the respective ketone product **84**, which after a reductive amination followed by a benzyl ether deprotection, afforded the crude hyacinthacine C-type analogue **86**. To purify **86**, Vankar *et al.* peracetylated the crude mixture, followed by its deprotection. After washing the residue with chloroform, the purified **86** was obtained.

2.11. Vankar *et al.* 2016

In continuation with synthetic efforts towards synthesizing different unnatural analogues of bicyclic iminosugars, Vankar *et al.* reported in 2016, a new strategy for accessing a large number of



Scheme 7. A total synthesis of the hyacinthacine C-type related analogue **86** by Vankar *et al.* [75].

complex hyacinthacine C_{2/3} related products [76]. Vankar and co-workers' synthesis of the unnatural hyacinthacine C-type alkaloids can be summarised in Scheme 8. Their synthetic work began with the oxidation of pyrrolidine **87** to its corresponding aldehyde using a CrO₃-pyridine-Ac₂O reagent system. The aldehyde formed was then treated with allylzinc bromide to afford compound **88** which underwent *O*-benzylation to the product **89**. The olefin moiety of compound **89** was then subjected to a nonselective dihydroxylation with OsO₄ and NMO which returned an inseparable mixture of diols. A selective protection of the primary alcohol in each diol resulted in the corresponding silyl ethers **90** and **91**, which were readily separable. To establish the methodology, Vankar and co-workers quantitatively mesylated the free hydroxy group in compound **90**. Without purification, the mesylated **90** then underwent an *N*-Boc deprotection to the corresponding amine salt, which under basic conditions furnished pyrrolizidine **92**. A global deprotection, followed by peracetylation as a means of purification afforded the pentaacetylate **94**. After a comprehensive structural

analysis, the pentaacetylate **94** was deprotected using aqueous ammonia in methanol which gave the final pentahydroxylated hyacinthacine C_{2/3}-type analogue **96**. Having established this synthetic route, Vankar and co-workers then employed a similar reaction scheme to diastereomer **91** which successfully converted into compound **97**. Vankar *et al.* also readily obtained two more epimers, **107** and **108**, from subjecting pyrrolizidine **98** to the same sequence of reactions. The only notable difference is that the hydroxy group of olefin **99** was protected as the acetate **100** instead of the benzyl ether. Of the four hyacinthacine C_{2/3} products synthesized, none matched the putative structure for hyacinthacine C₃, but they certainly provided a better understanding of the access to these complex iminosugars. Additionally, the biological activities of these final products were evaluated as potential glycosidase inhibitors against a panel of eight commercially available enzymes. The hyacinthacine analogues **96** and **107** displayed selective activity against jack bean α -mannosidase (IC₅₀ = 81.2 μ M and IC₅₀ = 95 μ M, respectively) with analogue **107** also displaying moderate activity

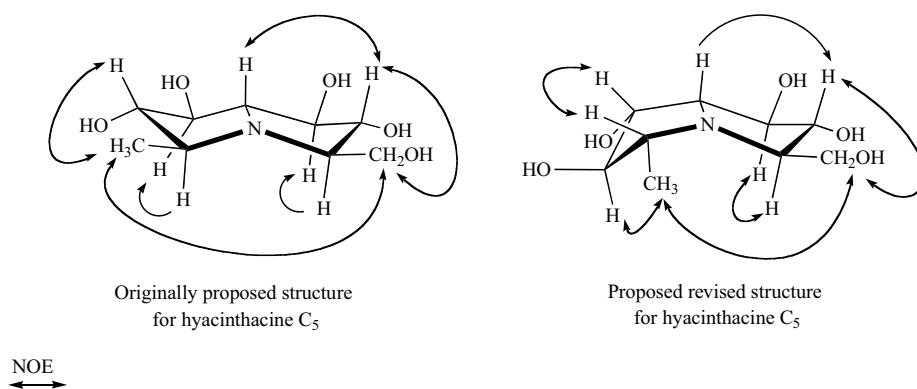


Fig. (8). The NOE signals reported for the putative hyacinthacine C₅ (left) along with the revised NOE signals proposed by Goti *et al.* [50, 63].

against coffee bean α -galactosidase (IC₅₀ = 120 μ M). Final remarks from the authors suggested that given these activities, hyacinthacine analogues **96** and **107** are potential anti-diabetic and anti-cancer leads, respectively.

2.12. Goti *et al.* 2017

A recent addition towards the synthesis of the hyacinthacine C-type alkaloids was reported in 2017 by Goti and co-workers through their synthesis of the proposed structure of (+)-hyacinthacine C₅ and its C-5 epimer **120** [63]. Their goal was to implement a straightforward and convenient synthesis of these complex iminosugar motifs. To access both these structures, their convergent synthesis began with the preparation of both the arabinose-derived cyclic nitrone **109** and the racemic lithiated 3-methyl-substituted benzyloxyallene **110** (Scheme 9). The lithiated derivative **110** was then added to nitrone **109** to form intermediate **111**, which upon standing at room temperature for 24 hours, cyclized to afford a 1:1 separable mixture of 1,2-oxazines **112** and **113**. Both oxazines were then individually subjected to hydroboration which delivered respective alcohols **114** and **115**. Goti and co-workers were initially focused on establishing the synthetic pathway towards the proposed structure of (+)-hyacinthacine C₅ and so their attention turned to alcohol **114** which was protected to give the penta-benzylated product **116**. Next, reductive amination of **116** with samarium diiodide in THF cleaved the N-O bond to give the amino-alcohol, which was subsequently converted to pyrrolizidine **118** via mesylation of the primary hydroxy group, then spontaneous cyclization. Finally, all benzyl ether groups were removed *via* hydrogenolysis to afford the purported (+)-hyacinthacine C₅. Using a similar method, 5-*epi*-hyacinthacine C₅ **120** was synthesized after alcohol **115** underwent a reductive amination followed by bis-mesylation of the hydroxy groups which concurrently cyclized to give pyrrolizidine **119**. After a LiAlH₄ reduction of the sulfonyl group on **119**, the corresponding alcohol was subjected to hydrogenolysis to give the globally deprotected pyrrolizidine, 5-*epi*-hyacinthacine C₅ **120**.

As per the previous reports by Yu *et al.* [61] and Tamayo *et al.* [62], Goti and co-workers also found that the configuration of the putative structure of hyacinthacine C₅ was incorrect. Neither structure synthesized in this report matched the spectroscopic data of the natural isolate. Despite being unable to resolve the true structure of hyacinthacine C₅, Goti *et al.* revised the originally reported NOE enhancements for the natural isolate and after finding similar NOE correlations in their synthetic products, were able to suggest an alternative configuration which could be appropriately labelled as 1-*epi*-hyacinthacine C₄ (Fig. 8).

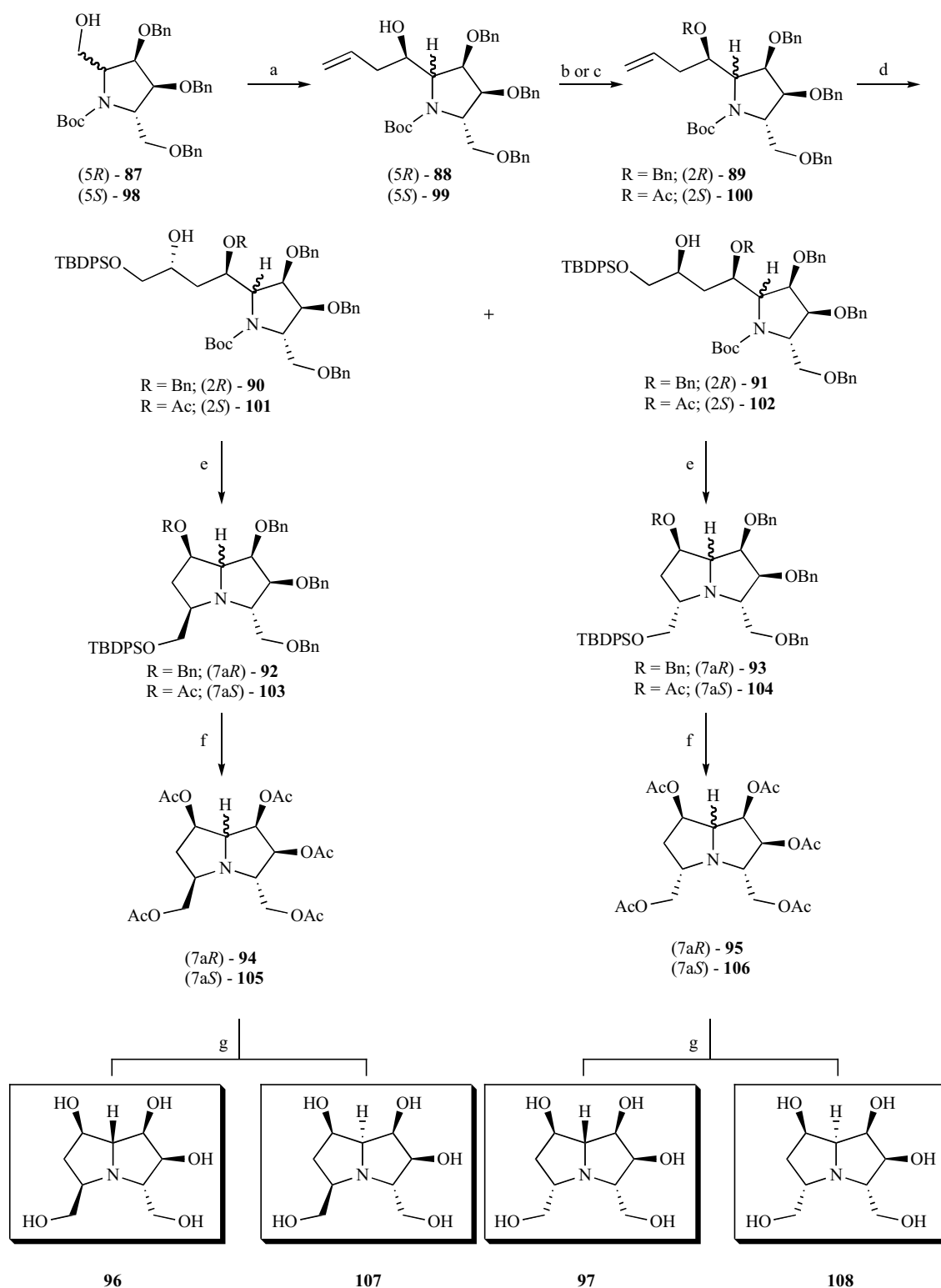
2.13. Pyne *et al.* 2018

Pyne and co-workers employed a flexible synthesis which is the latest addition towards accessing the hyacinthacine C-type alkaloids

[64]. This synthetic work resulted in a total of seven hyacinthacine C-type analogues including the total synthesis of natural (+)-hyacinthacine C₅ **141** and confirmed the hypothesis proposed by Goti *et al.* that its true configuration is that of (+)-1-*epi*-hyacinthacine C₄. In this synthesis, Pyne *et al.* used a key Petasis borono Mannich reaction at one stage to obtain the L-xylose derived *anti*-1,2-amino alcohol **121** (Scheme 10). After protecting the secondary amine with di-*tert*-butyl decarbonate to afford **122**, the product was then converted to the oxazolidinone **123**. Compound **123** was then subjected to ring closing metathesis (RCM) reaction conditions which afforded the bicyclic alkene **124** in 85% yield. The olefin moiety of compound **124** was then subjected to a dihydroxylation reaction with K₂OsO₄·H₂O and NMO which afforded a 3:1 mixture of diastereomers **125a** and **125b**, respectively which only separated efficiently after conversion to their acetonides **126** and **127**, respectively. To establish the methodology, acetonide **126** was hydrolysed with base under microwave conditions to afford amino diol **128**, which was subsequently converted to the pyrrolizidine **129** in high yield after employing Appel cyclization conditions [77]. A global deprotection of pyrrolizidine **129** over PdCl₂ followed by basic-ion exchange chromatography resulted in the (+)-5,6-di-*epi*-hyacinthacine C₅ **130**. Using similar methodology, hyacinthacine C-type analogue (–)-5,7-di-*epi*-hyacinthacine C₅ **133** was also obtained except that its related pyrrolizidine core **132** was formed via selective *O*-mesylation, then heat induced cyclization.

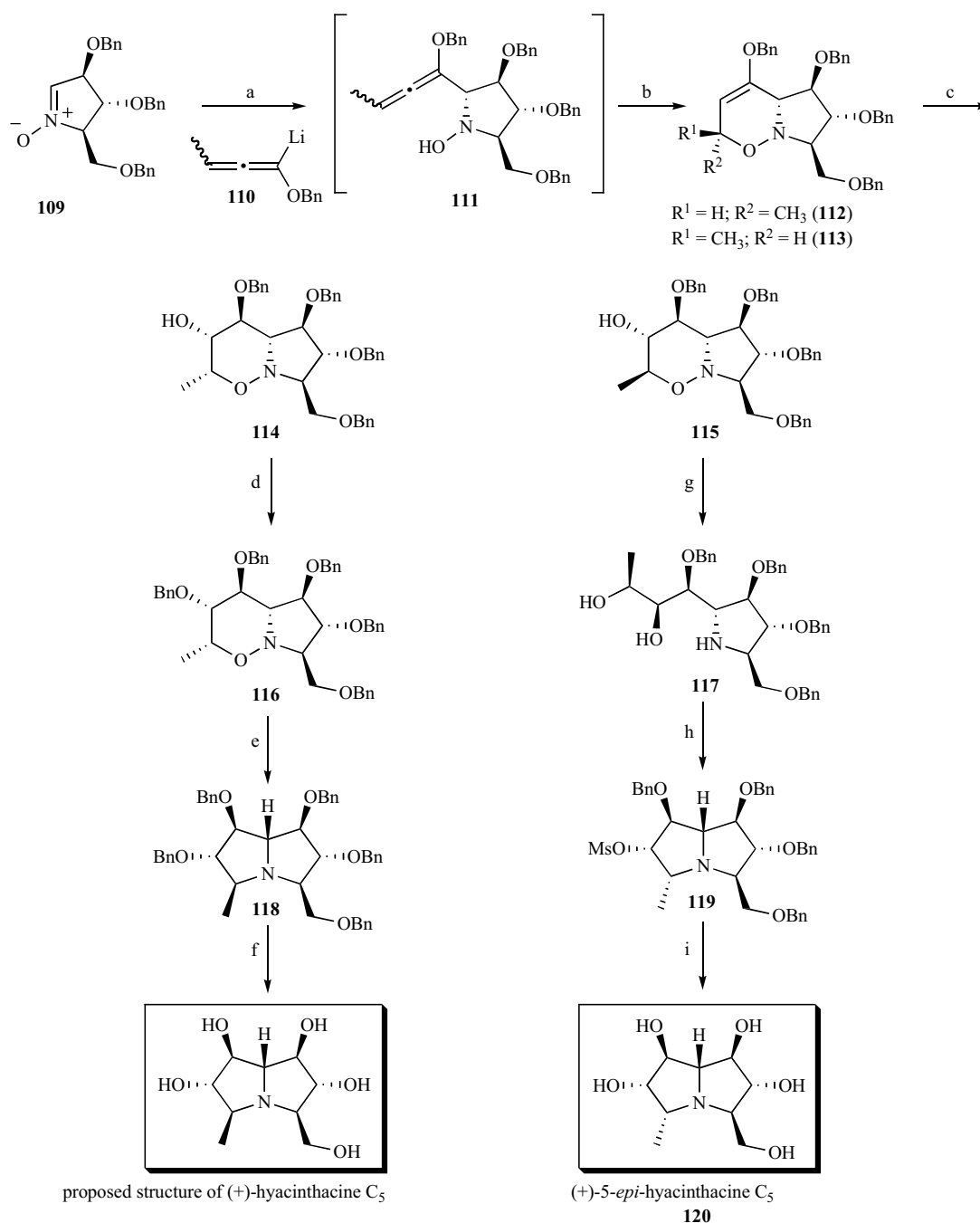
Attention was then focused on manipulating the stereoconfiguration of the C6, C7 hydroxy groups without affecting the stereochemistry of the A-ring in the pyrrolizidine core (Scheme 10). To explore this chemistry, the C8 hydroxy group in pyrrolizidine **129** was *O*-benzylated and then acid hydrolysed which returned diol **135**. Diol **135** was then converted to its cyclic sulfate **136** followed by a subsequent ring opening with CsOBz (prepared *in situ* from Cs₂CO₃ and BzOH) which gave an inseparable 1:4 mixture of regioisomers **137a** and **137b**, respectively. The mixture was subjected to base mediated hydrolysis of the benzoate in **137a** and **137b** which returned isolatable diols **140** and **138**, respectively. Diols **138** and **140** were individually debenzylated under hydrogenolysis conditions (over PdCl₂), which returned pure samples of both (+)-5-*epi*-hyacinthacine C₅ **139** and (+)-1-*epi*-hyacinthacine C₄ **141**, respectively.

Having obtained four hyacinthacine C-type alkaloids, Pyne *et al.* then applied the same sequence of reactions and conditions to a 1:2 mixture of the amino diols **121** and **142**, respectively (Scheme 11). From the 1'*S*-**142**, they secured the putative structure of (+)-hyacinthacine C₅, along with (–)-7-*epi*-hyacinthacine C₅ **149** and (+)-6,7-di-*epi*-hyacinthacine C₅ **158**. The spectroscopic data of all final products were compared against the spectroscopic data of the natural hyacinthacine C₁, C₄ and C₅ alkaloids. It was discovered



Reagents and conditions: (a) (i) CrO₃, pyridine, Ac₂O, CH₂Cl₂, 0°C; (ii) AllylBr, Zn, satd NH₄Cl, 85% (**88** over two steps), 78% (**99** over two steps); (b) BnBr, NaH, TBAI (cat.), THF, 90% (**89**); (c) Ac₂O, Et₃N, DMAP, CH₂Cl₂, 87% (**100**); (d) (i) OsO₄, NMO, acetone/H₂O/*t*-BuOH (5:1:1); (ii) TBDPSCl, Et₃N, DMAP, 50% (**90** over two steps), 38% (**91** over two steps), 63% (**101** over two steps), 26% (**102** over two steps); (e) (i) Et₃N, MsCl, DMAP; (ii) TFA; (iii) Et₃N, THF, 70°C, 45% (**92** over three steps), 40% (**93** over three steps), 46% (**103** over three steps), 47% (**104** over three steps); (f) (i) H₂, Pd(OH)₂/C, MeOH; (ii) Ac₂O, pyridine, 80% (**94** over two steps), 80% (**95** over two steps), 85% (**105** over two steps), 85% (**106** over two steps); (g) aq. NH₃/MeOH (1:3), 89% (**96**), 78% (**97**), 89% (**107**), 90% (**108**).

Scheme 8. A stereodivergent synthesis of hyacinthacine C₂₃-related analogues **96**, **107**, **97** and **108** by Vankar *et al.* [76].

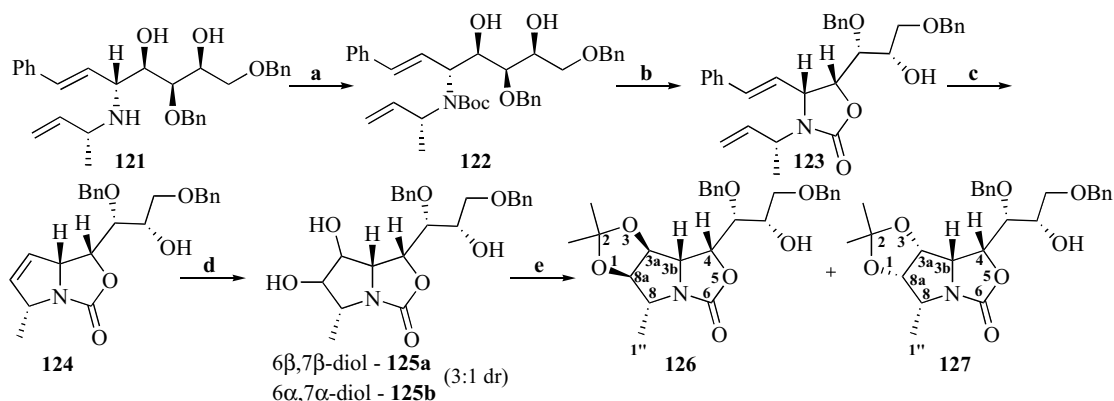


Reagents and conditions: (a) **110**, THF, -78 °C; (b) CH₂Cl₂, 38% (**112** over two steps), 36% (**113** over two steps); (c) BH₃·THF, THF, -30 °C, then H₂O₂, NaOH, 57% (**114**), 41% (**115**); (d) NaH, BnBr, TBAI (cat.), THF, 39%; (e) (i) SmI₂, THF; (ii) Et₃N, MsCl, CH₂Cl₂, 73% (two steps); (f) H₂, 10% Pd/C, HCl, MeOH, quant.; (g) SmI₂, THF, 96%; (h) Et₃N, MsCl, CH₂Cl₂, 80%; (i) (i) LiAlH₄, THF, reflux, 74%; (ii) H₂, 10% Pd/C, HCl, MeOH, 69%.

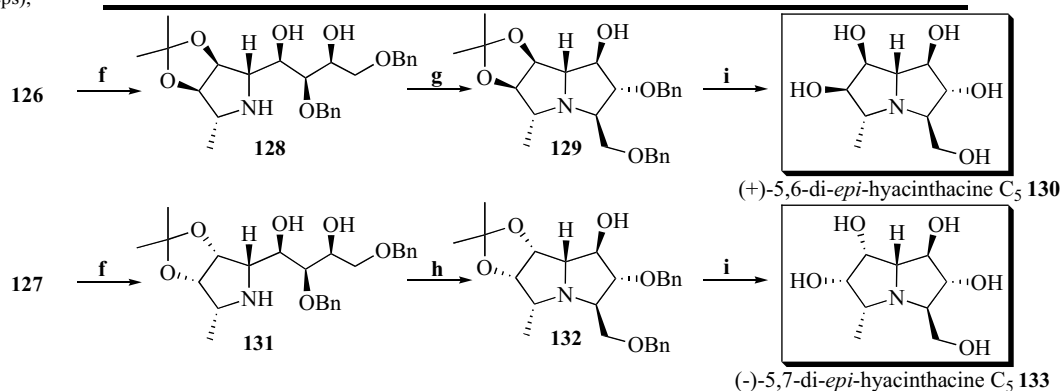
Scheme 9. The total synthesis of the proposed structure of (+)-hyacinthacine C₅ and 5-*epi*-hyacinthacine C₅ **120** by Goti *et al.* [63].

that (+)-1-*epi*-hyacinthacine C₄ **141** matched both ¹H NMR and ¹³C NMR spectroscopic data and had similar specific optical rotations when compared with the natural isolate labelled hyacinthacine C₅. The authors found that their spectroscopic data for synthetic (+)-hyacinthacine C₅ [61-63], (-)-7-*epi*-hyacinthacine C₅ [61] **149** and (+)-6,7-di-*epi*-hyacinthacine C₅ [62] **158** confirmed the proposed structures and absolute configurations of the literature reported synthetic products (+)-hyacinthacine C₅, (-)-7-*epi*-hyacinthacine C₅ **49** and (+)-6,7-di-*epi*-hyacinthacine C₅ **64**, respectively. However, when they compared their synthetic (+)-5-*epi*-hyacinthacine C₅ **139**

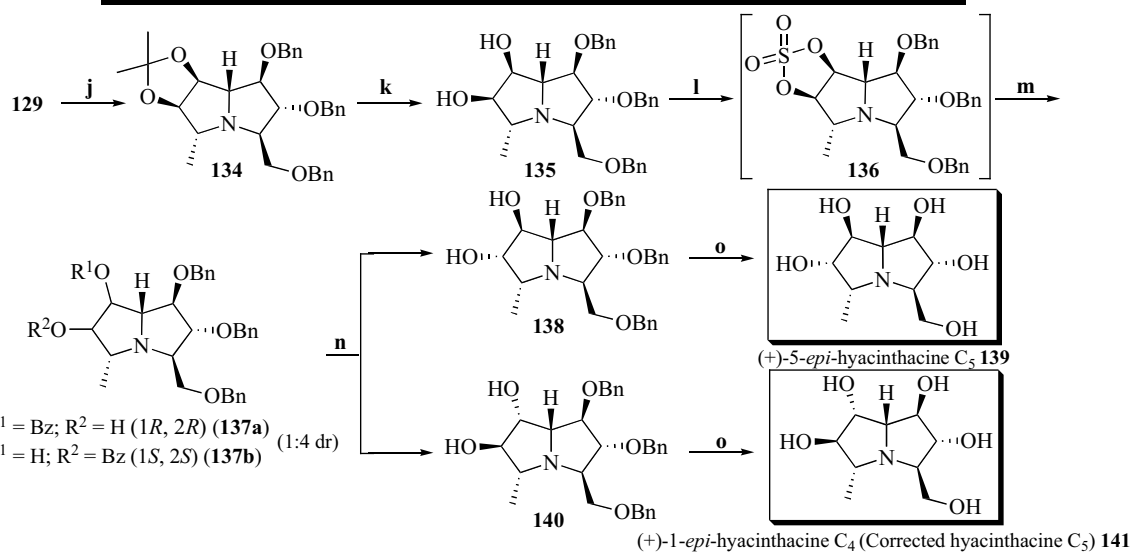
against the same compound **120** reported by Goti *et al.*, Pyne and co-workers found several spectroscopic differences that led them to conclude that they were not the same product. To try and resolve this issue, they extensively characterised the peracetylated derivative of (+)-5-*epi*-hyacinthacine C₅ **139** as well as its HCl salt, although neither were a spectroscopic match to the product proposed by Goti *et al.* Pyne acknowledged the possibility of a double inversion of amino diols **128**, **131** or **147** after *O*-mesylation, and so would retain stereochemistry in the corresponding pyrrolizidine structures. Given their structures for



Reagents and conditions: (a) (Boc)₂O, DIPEA, DCE, reflux, 62%; (b) NaH, THF, 65°C, 85%; (c) Grubbs II cat. (5 mol %), CH₂Cl₂, reflux, 85%; (d) K₂OsO₄·H₂O, aq NMO, acetone/H₂O, 35°C, 78% (**125a**, **125b** (3:1)); (e) H₂SO₄, CuSO₄, acetone, 71% (**126** over two steps), 24% (**127** over two steps);



Reagents and conditions: (f) NaOH, EtOH, Microwave, 110°C, 86% (**128**), 87% (**131**); (g) PPh₃, CBr₄, Et₃N, CH₂Cl₂, 0°C, 87%; (h) (i) MsCl, Et₃N, 0°C; (ii) DCE, 80°C, 87% (**132**); (i) H₂, 5% PdCl₂, MeOH, then basic ion exchange, 88% ((+)-5,6-di-epi-hyacinthacine C₅ **130**), 98% ((-)-5,7-di-epi-hyacinthacine C₅ **133**).

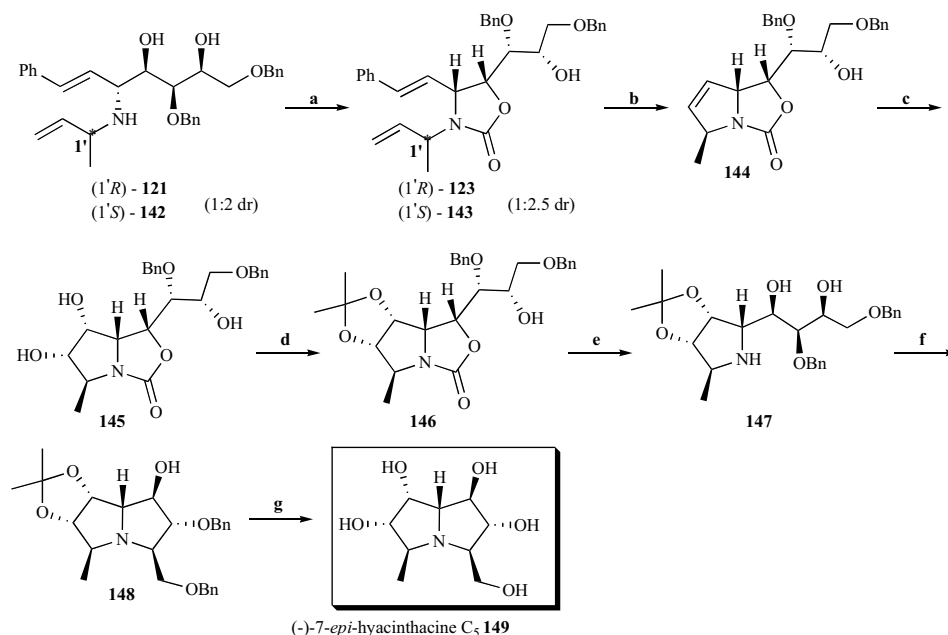


Reagents and conditions: (j) BnBr, NaH, TBAI (cat.), THF, reflux, 89%; (k) HCl, EtOH/H₂O, 98%; (l) SO₂Cl₂, Et₃N, CH₂Cl₂; (m) (i) BzOH, Cs₂CO₃, DMSO, 50°C; (ii) H₂SO₄/THF/H₂O (1:8:3), 60°C, 49% (**137a**, **137b** (1:4)); (n) K₂CO₃, MeOH, 37% (**140** over two steps), 9% (**141** over two steps); (o) H₂, 5% PdCl₂, MeOH, then basic ion exchange, 95% ((+)-5-epi-hyacinthacine C₅ **139**), 95% ((+)-1-epi-hyacinthacine C₄ **141**), 75%.

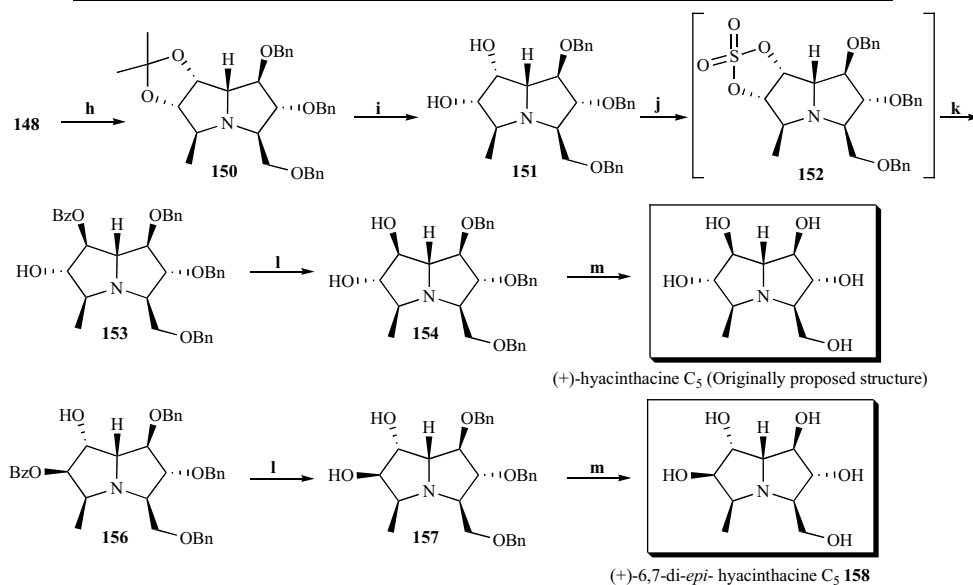
Scheme 10. The total synthesis of (+)-5,6-di-epi-hyacinthacine C₅ **130**, (-)-5,7-di-epi-hyacinthacine C₅ **133**, (+)-5-epi-hyacinthacine C₅ **139** and (+)-1-epi-hyacinthacine C₄ **141** [64].

synthetic (+)-hyacinthacine C₅, **149** and **158** were consistent with the previously reported structures for (+)-hyacinthacine C₅ [61], (-)-7-epi-hyacinthacine C₅ [61] **49** and (+)-6,7-di-epi-hyacinthacine C₅ [62] **64**, they were confident this did not occur. In contrast, Goti and co-workers successfully synthesised the proposed structure of

hyacinthacine C₅ but did not employ the same conditions to obtain its C₅ epimer. Instead, they cyclized the bis-mesylated amino product **117** and so there is the possibility that cyclization could afford the stable 4-membered bicyclic *N*-heterocycle [78]. This issue has not yet been resolved and so highlights the difficulty often



Reagents and conditions: (a) triphosgene, Et₃N, CH₂Cl₂, 43% (**123**, **143** (1:2.5)); (b) Grubbs II cat. (5 mol %), CH₂Cl₂, reflux, 53%; (c) K₂O₄·H₂O, aq NMO, acetone/H₂O, 35 °C, 98%; (d) H₂SO₄, CuSO₄, acetone, 98%; (e) NaOH, EtOH, μW, 110 °C, 84%; (f) (i) MsCl, Et₃N, 0 °C, 84%; (ii) DCE, 80 °C, 76%; (g) H₂, 5% PdCl₂, MeOH, then basic-ion exchange, 99% ((-)-7-*epi* hyacinthacine C₅ (**149**)).



Reagents and conditions: (h) BnBr, NaH, TBAI (cat.), THF, reflux, 87%; (i) HCl, EtOH/H₂O, 85%; (j) SO₂Cl₂, Et₃N, CH₂Cl₂; (k) (i) BzOH, Cs₂CO₃, DMSO, 50 °C; (ii) H₂SO₄/THF/H₂O (1:8:3), 60 °C, 61% (**153**), 12% (**156**); (l) K₂CO₃, MeOH, 79% (**154**), 90% (**157**); (m) H₂, 5% PdCl₂, MeOH, then basic-ion exchange, 99% ((+)-hyacinthacine C₅), 78% ((+)-6,7-di-*epi*-hyacinthacine C₅ (**158**)).

Scheme 11. The total synthesis of (-)-7-*epi*-hyacinthacine C₅ **149**, (+)-hyacinthacine C₅, (+)-6,7-di-*epi*-hyacinthacine C₅ **158** [64].

associated with assigning the structures of the hyacinthacine C-type alkaloids. Further investigation is certainly required.

Despite this, Pyne and co-workers evaluated the final products as potential glycosidase inhibitors. They found that **130** and **133** showed weak inhibition of the α-glucosidase of rat intestinal isomaltase (IC₅₀ values of 20 μM and 42 μM, respectively) and moderate inhibition against the α-glucosidase of rat intestinal sucrase (IC₅₀ values of 13 μM and 9.9 μM, respectively). Compound **130** proved to be a weak inhibitor against yeast α-glucosidase (IC₅₀ value of 83 μM) and rat intestinal maltase (IC₅₀ value of 61 μM) but showed moderate activity against amylglucosidases from *A. niger* and *Rhizopus* sp (IC₅₀ values of 25

μM and 16 μM, respectively). Compound **149** seemed relatively inactive but compound **139** displayed moderate inhibition against α-glucosidases of rat intestinal maltase and human lysosome (IC₅₀ values of 21 μM and 15 μM, respectively) and weak inhibition against the amylglucosidase of *A. niger* (IC₅₀ value of 41 μM). Besides **139**, only the putative (+)-hyacinthacine C₅ was noted to display a weak inhibition against the α-L-rhamnosidase of *Penicillium decumbens* (IC₅₀ value of 74 μM). Pyne and co-workers noted a correlation between the orientation of the C5 methyl group and the IC₅₀ against each enzyme tested. More specifically, if the hyacinthacine C-type alkaloid contained an (5*S*)-methyl then the IC₅₀ values increased with consequently a reduction in the

selectivity and potency of the compound as a glycosidase inhibitor. Comparatively, for a decrease in the IC₅₀, the hyacinthacine C-type alkaloids containing an (5*R*)-methyl proved more suitable candidates as potential glycosidase inhibitors.

CONCLUSION

Research towards pyrrolizidine alkaloid isolation and synthesis is of paramount importance. From an economic point of view, characterisation of these compounds is essential to prevent illness occurring in livestock and more importantly, humans. The polyhydroxylated pyrrolizidine alkaloids containing a C-3 methyl or hydroxymethyl represent only a slight fraction of the ever-growing number of discovered pyrrolizidine alkaloids but are of great importance as they are potentially medicinally useful. Of these, the hyacinthacine C-type alkaloids have generated recent interest which can be attributed to their inherent glycosidase inhibitory activities, making this class of compound suitable candidates for anti-diabetic, anti-obesity, anti-cancer and in some cases, anti-bacterial drug scaffolds. Their high degree of oxidation in conjunction with complex stereochemistry presents as a rewarding synthetic feat for any laboratory prepared to undertake their synthesis and is beginning to rapidly grow as a research area. Additionally, a number of mistakes associated with their structures warrant their synthesis as a way to confirm or correct their proposed structures. To date, methods of total synthesis have confirmed the structure of hyacinthacine C₂ to be correct but have found that the structure for hyacinthacine C₃ requires revision. The structures for hyacinthacine C₁ and hyacinthacine C₄ remain insecure since they both contain different spectroscopic data but have been assigned the same relative stereochemistry. Pyne *et al.* recently confirmed the hypothesis by Goti and co-workers, by synthesizing the correct structure for hyacinthacine C₅ found to be (+)-1-*epi*-hyacinthacine C₄. However, their spectroscopic data for (+)-5-*epi*-hyacinthacine C₅ **139** when compared against the same compound **120** reported by Goti *et al.*, did not match, requiring further investigation. Despite these issues, the studies reported in this review have resulted in the synthesis of important regio- and stereo-isomers of the hyacinthacine C-type compounds and so presents as a great significance to the large community interested in the preparation of glycosidase inhibiting and consequent therapeutic iminosugars. Unfortunately, the glycosidase activities of a number of these analogues remain uncertain as they were not evaluated for their biological activity. This observation suggests the need for an accessible approach for assaying these compounds as potential drug motifs for the aforementioned illnesses and complications. Although they may not ever be marketable drugs, synthetic access and glycosidase testing of the hyacinthacine C-type alkaloids represent a positive step towards understanding the structure-activity-relationship of a glycosidase active site mechanism. This foundation prepares the knowledge base required to eventually design marketable drugs that manage and selectively treat the aforementioned illnesses with minimal side effects.

CONSENT FOR PUBLICATION

Not applicable.

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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Declared none.

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