**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 µM) 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.

**Keywords:** Iminosugars, pyrrolizidine, hyacinthacine, alkaloids, total synthesis, natural product, glycosidase inhibition.

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].

![General structure for pyrrolizidine alkaloids](image_url)

**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
were also isolated from the bark of *Casuarina equisetifolia* L because of its traditional medicinal uses including the management of diabetes and bacterial infections [28]. Although later, its C-7α 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.

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 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. (3), 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.

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.

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 Mytaceae 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 pyrrolidine 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 displayed both weak inhibition of almond β-glucosidase (IC50 values of 320 µM and 100 µM, respectively) and bovine liver β-galactosidase (IC50 values of 110 µM and 160 µM, respectively). Both alkaloids also showed selective inhibition for rat intestinal lactase β-galactosidase, however, hyacinthacine B2 proved far more active (IC50 = 3.6 µM) than hyacinthacine B1 (IC50 = 270 µM). Interestingly, hyacinthacine C1 proved moderately active against the amyloglucosidase of *Aspergillus niger* (IC50 = 84 µM), 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 µM, 73 µM, 160 µM and 18 µM, respectively) and also *A. niger* amyloglucosidase (IC50 values of 25 µM, 8.6 µM, 17 µM and 51 µM, respectively). Hyacinthacine A1 proved to be the only alkaloid active against rat epididymis α-L-fucodisase (IC50 = 46 µM) and also rice α-glucosidase (IC50 = 240 µM). Hyacinthacine A1 and A2 also displayed activity against almond β-glucosidase with IC50 values of 250 µM and 150 µM, 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 A1, 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, A7, B4, and B6, where they showed inhibitory activity towards the amyloglucosidase of *A. niger* with IC50 values of 110 µM, 89 µM and 110 µM, respectively. In addition to this, hyacinthacine B4 displayed a moderate inhibition of bovine epididymis α-L-fucodisase with an IC50 value of 23 µM [48].

Two years later, Asano and co-workers reported findings of long side chain pyrrolidines 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 alkald [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, 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 µM and 6.6 µM, 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. saccharoyticum* β-glucosidase with IC50 values of 9.5 µM, 25.4 µM and 5.1 µM, 11.4 µM, 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 B2, C2, C3, C4 and C5 were isolated and tested against a panel of selected glycosidases. Of these results, hyacinthacines C2, C5 and C3 proved noteworthy activity against *C. saccharoyticum* β-glucosidase with IC50 values of 13 µM, 25 µM and 48 µM, respectively. Hyacinthacine C5 proved to be the only active compound against rice α-glucosidase (IC50 = 110 µM), but shared similar activities with hyacinthacine C3 against rat α-glucosidase (IC50 = 45 µM and 77 µM, respectively) and the amyloglucosidase of *A. niger* (IC50 = 57 µM for both C2 and C3). Hyacinthacine C3 displayed a unique inhibition of bovine liver β-glucosidase with an IC50 value of 52 µM, whilst hyacinthacine C2 proved the only alkaloid capable of inhibiting human placent a α-L-fucodisase with an IC50 value of 17 µM [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 1H,1H coupling
| enzyme                        | IC<sub>50</sub> (µM)       |          |          |          |          |
|------------------------------|----------------------------|----------|----------|----------|----------|
|                              | Hyacinthacine A<sub>1</sub> | Hyacinthacine A<sub>2</sub> | Hyacinthacine A<sub>3</sub> | Hyacinthacine A<sub>4</sub> |          |
| α-glucosidase                |                            |          |          |          |          |
| Yeast                        | .<sup>a</sup>              | -        | -        | -        | NI       |
| Rice                         | 240                        | NI       | NI       | NI       | NI       |
| Rat intestinal maltase       | NI<sup>b</sup>             | NI       | NI       | NI       | NI       |
| Rat liver lysosome           | -                          | -        | -        | -        | -        |
| β-glucosidase                |                            |          |          |          |          |
| Almond                       | 250                        | 150      | NI       | -        |          |
| Caldocellum saccharolyticum  | -                          | -        | -        | 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             |                            |          |          |          |          |
| Aspergillus niger            | 25                         | 8.6      | 17       | NI       |          |
| α-L-rhamnosidase             |                            |          |          |          |          |
| Penicillium decumbens        | -                          | -        | -        | NI       |          |

<sup>a</sup>: Not tested, <sup>b</sup>NI: No inhibition (less than 50% inhibition at 1000 µM)
| Enzyme                  | Hyacinthacine A₅ | Hyacinthacine A₆ | Hyacinthacine A₇ | Hyacinthacine B₁ |
|------------------------|------------------|------------------|------------------|------------------|
| 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              |
| Caldocellum saccharolyticum | 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               |
| α-L-rhamnosidase       |                  |                  |                  |                  |
| Porcine kidney         | -                | -                | -                | NI               |
| Amyloglucosidase       |                  |                  |                  |                  |
| Aspergillus niger      | 110              | NI               | NI               | NI               |
| α-L-rhamnosidase       |                  |                  |                  |                  |
| Penicillium decumbens  | NI               | NI               | NI               | -                |

Table 1. contd...
| Enzyme                  | Hyacinthacine B₂ | Hyacinthacine B₃ | Hyacinthacine B₄ | Hyacinthacine B₅ |
|------------------------|------------------|------------------|------------------|------------------|
| **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               | -                | -                |
| Caldacellum saccharolyticum | 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               | -                | -                |
| **α-L-rhamnosidase**   |                  |                  |                  |                  |
| *Penicillium decumbens* | -              | -                | NI               | NI               |

Table 1. contd...
| Enzyme               | Hyacinthacine B₁ | Hyacinthacine B₂ | Hyacinthacine C₁ (1999) | Hyacinthacine C₂ |
|----------------------|-------------------|-------------------|--------------------------|------------------|
| α-glucosidase        | Yeast             | NI                | -                        | -                |
|                      | Rice              | NI                | NI                       | NI               |
|                      | Rat intestinal maltase | NI        | NI                       | NI               |
|                      | Rat liver lysosome | NI                | -                        | -                |
| β-glucosidase        | Almond            | -                 | -                        | NI               |
|                      | Caldoccum saccharolyticum | NI        | NI                       | NI               |
|                      | Human placenta    | -                 | -                        | -                |
| α-galactosidase      | Coffee beans      | -                 | NI                       | -                |
|                      |                   |                   | NI                       | -                |
| β-galactosidase      | Bovine liver      | NI                | NI                       | NI               |
|                      | Rat intestinal lactase | -           | -                        | NI               |
|                      | Rat epididymis    | -                 | -                        | -                |
| α-mannosidase        | Jack bean         | -                 | 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     | Aspergillus niger | NI                | 270                      | 84               |
|                      |                   |                   |                          | 550              |
| α-L-rhamnosidase     | Penicillium decumbens | NI        | -                        | -                |

Table 1. contd...
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Current Organic Synthesis, 2019, Vol. 16, No. 4  505

| Enzyme                  | Hyacinthacine C₃ | Hyacinthacine C₄ (2007) | Hyacinthacine C₅ | α-5-C-(3-hydroxybutyl)hyacinthacine A₁ |
|-------------------------|------------------|--------------------------|------------------|-------------------------------------|
| α-glucosidase           |                  |                          |                  |                                     |
| Yeast                   | -                | -                        | -                | NI                                  |
| Rice                    | NI               | 110                      | NI               | NI                                  |
| Rat intestinal maltase  | NI               | 45                       | 77               | NI                                  |
| Rat liver lysosome      | -                | -                        | -                | -                                   |
| β-glucosidase           |                  |                          |                  |                                     |
| Almond                  | -                | -                        | -                | 49                                  |
| Caldocellum saccharolyticum | 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          | -                | -                        | -                | -                                   |
| α-L-rhamnosidase        |                  |                          |                  |                                     |
| Penicillium decumbens   | -                | -                        | -                | NI                                  |

Table 1. contd…
| Enzyme                      | α-5-C-(1,3-dihydroxybutyl)hyacinthacin A1 | α-5-C-(1,3,4-trihydroxybutyl)hyacinthacine A1 | α-5-C-(3-hydroxybutyl)hyacinthacine A2 | 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                                      |
| *Caldocellum saccharolyticum* | 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            | -                                         | -                                             | -                                      | -                                       |
| α-amylglucosidase         |                                            |                                               |                                        |                                         |
| *Aspergillus niger*       | -                                         | -                                             | 180                                    | -                                       |
| α-L-rhamnosidase          |                                            |                                               |                                        |                                         |
| *Penicillium decumbens*   | 180                                       | NI                                            | -                                      | NI                                      |
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 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].

To date, fourteen of the natural hyacinthacines, A₁, A₂, A₃, A₄, A₇, B₁, B₂, B₃, B₄, B₅, C₁, C₂, C₃ and C₄ 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 A₃ through methods of total synthesis [61, 63, 65]. To the best of our knowledge, hyacinthacine A₃ 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 B₁, C₁ 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 C₁ 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-pyrrolidino-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 acids 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 C₅ along with its epimer, 5-epi-hyacinthacine C₅ 10 in 72% and 71% yields, respectively. Having established the synthetic pathway to hyacinthacine C₅, 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.
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 C2/3 from the arabinose
The History of the Glycosidase Inhibiting Hyacinthacine C-type Alkaloids

Organic Synthesis, 2019, Vol. 16, No. 4 509

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 7α-epi-hyacinthacine C₁ [36] and 5,7α-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
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 NaBH₄ 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 pyrrolidinone cores. To both pyrrolidinone mixtures were then added MeONa in MeOH to facilitate a one-pot debenzyol- and deacetylation to afford triols 34 and 35, respectively. Final O-benzyl deprotection of 34 and 35 revealed the desired hyacinthacine C-type analogues, 7a-epi-hyacinthacine C₁ 36 and 5,7a-di-epi-hyacinthacine C₁ 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 C₅ 36 and 7-epi(-)-hyacinthacine C₅ from the L-arabinose derived cyclic nitrone 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 C₅. Their synthesis began with the addition of the lithiated dithiane 39 to the cyclic nitrone 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 separated using Zn-HOAc to afford the pyrrolizidines 43 and 44, respectively. Initially focusing on the enantiomer of hyacinthacine C₁, the dihiketol 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 debenzylation revealed the final product as (-)-hyacinthacine C₅. 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 dithiketol to give the corresponding ketone in 61% yield. A stereoselective reduction of the ketone with NaBH₄ 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 C₅ 49. Having established the synthetic pathway, Yu and co-workers successfully synthesized (-)-6-epi-hyacinthacine C₅ 52 by...
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 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-
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 C5-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, 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 C6, C7-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) TPAP, CH 2Cl 2, 4 Å MS, 95%; (b) Ph 3PCHCOMe, 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].
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 nitrones 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 nitrode 65 (Scheme 6). The cyclic nitrode 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 Sn1-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)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.

In the same year, Vankar and co-workers reported their synthetic work towards the synthesis of homoanalogues of hyacinthacine C3 [75]. Their 2014 report details starting from the arabinose-based cyclic nitrode 74 which underwent a 1,3-dipolar cycloaddition reaction with the D-mannitol-aldehyde derived dipolarphile 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 benzylolation 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-benzylolation of both hydroxy groups afforded 80, which upon removal of the cyclohexyldiene 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 MeMgl, 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.

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
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 CrO$_3$-pyridine-Ac$_2$O 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 OsO$_4$ 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 pyrrolidine 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 C$_3$, but they certainly provided a better understanding of the access to these complex iminosugars. Additionally, the biological activities the 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-mannosidae (IC$_{50} = 81.2$ µM and IC$_{50} = 95$ µM, respectively) with analogue 107 also displaying moderate activity.
against coffee bean α-galactosidase (IC₅₀ = 120 µM). 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 benzoxylpyrrolizidine 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 hydroxyl 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 C₅ (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-xylene 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 K₂OsO₅H₂O and NMO which afforded a 3:1 mixture of diastereomers 125a and 125b, respectively which only separated efficiently after conversion to their acetones 126 and 127, respectively. To establish the methodology, acetone 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 C₃ 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 CsOH (prepared in situ from Cs₂CO₃ and Br₂OH) 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 debenzyalted 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 C₃ 149 and (+)-6,7-di-epi-hyacinthacine C₃ 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

![Fig. (8). The NOE signals reported for the putative hyacinthacine C₁ (left) along with the revised NOE signals proposed by Goti et al. [50, 63].](image-url)
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), 20% (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].
that (+)-1-epi-hyacinthacine C₄ 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] and (+)-6,7-di-epi-hyacinthacine C₅ [62] confirmed the proposed structures and absolute configurations of the literature reported synthetic products (+)-hyacinthacine C₅, (−)-7-epi-hyacinthacine C₅ and (+)-6,7-di-epi-hyacinthacine C₅, respectively. However, when they compared their synthetic (+)-5-epi-hyacinthacine C₅ 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 as well as its HCl salt, although neither was 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  

**Scheme 9.** The total synthesis of the proposed structure of (+)-hyacinthacine C₅ and 5-epi-hyacinthacine C₅ by Goti et al. [63].
The previously reported structures for (+)-hyacinthacine C₅ [61], (–)-hyacinthacine C₄ [62] and co-workers successfully synthesised the proposed structure of synthetic (+)-hyacinthacine C₅. In contrast, Goti and co-workers synthesized the bis-mesylated amino ester of (–)-hyacinthacine C₄ [64], and so there is the possibility that cyclization could afford the stable 4-membered bicyclic product but did not employ the same conditions to obtain its C₅ epimer. Instead, they cyclized the bis-mesylated amino ester of (–)-5,7-di-epi-hyacinthacine C₅, 95% ((+)-5,6-di-epi-hyacinthacine C₅ 130), 98% ((–)-5,7-di-epi-hyacinthacine C₅ 133).
The History of the Glycosidase Inhibiting Hyacinthacine C-type Alkaloids

Organic Synthesis, 2019, Vol. 16, No. 4 519

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 (5S)-methyl then the IC50 values increased with consequently a reduction in the

Scheme 11. The total synthesis of (−)-7-epi-hyacinthacine C5, (+)-hyacinthacine C5, (+)-6,7-di-epi-hyacinthacine C5 [64].
selectivity and potency of the compound as a glycosidase inhibitor. Comparatively, for a decrease in the IC50, the hyacinthacine C-type alkaloids containing (5R)-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 C4 found to be (+)-1-epi-hyacinthacine C4. However, their spectroscopic data for (+)-5-epi-hyacinthacine C3 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 assessing 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 is of paramount importance. From an economic point of view, methods of total synthesis have confirmed the structures. To date, methods of total synthesis have confirmed the structure of 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 C4 found to be (+)-1-epi-hyacinthacine C4. However, their spectroscopic data for (+)-5-epi-hyacinthacine C3 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 assessing 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 is of paramount importance.
The History of the Glycosidase Inhibiting Hyacinthacine C-type Alkaloids

Current Organic Synthesis, 2019, Vol. 16, No. 4 521

Fellows, L.E.; Evans, S.V.; Nash, R.J.; Bell, E.A. Polyhydroxy plant alkaloids as glycosidase inhibitors and their possible ecological role. ACS Symp. Ser., 1986, 296, 72-78.

Nash, R.J.; Thomas, P.J.; Wagh, R.D.; Fleet, G.W.J.; Wormald, M.R.; de Q. Lilley, P.M.; Watkin, D.J. Casuarine: A very highly oxygenated pyrrolizidine alkaloid. Tetrahedron Lett., 1994, 35, 7849-7852.

Chopra, R.N.; Nayar, S.L. Glossary of Indian Medicinal Plants. Council of Scientific and Industrial Research: New Delhi, 1956.

Wormald, M.; Nash, R.; Watson, A.; Bhadoria, B.; Langford, R.; Sims, M.; Fleet, G. Casuarine-6-O-glucoside from Casuarina equisetifolia and Eugenia jambolana. Carbohydry. Lett., 1992, 2, 169-174.

Davis, A.P.; Payne, S.G.; Skelton, B.W.; White, A.H. Synthesis of Putative Uniflorine A. J. Org. Chem., 2004, 69, 3139-3143.

Matsumura, T.; Kasai, M.; Hayashi, T.; Ariawa, M.; Momose, Y.; Arii, I.; Amagaya, S.; Komatsu, Y. α-Glucosidase inhibitors from paraguayan natural medicine, nagapary, the leaves of Eugenia uniflora. Phar. Biol., 2000, 38, 302-307.

Rithiwigrom, T.; Pyne, S.G. Synthesis of (+)-uniflorine A: A structural reassessment and a configurational assignment. Org. Lett., 2008, 10, 2769-2771.

Davis, A.S.; Rithiwigrom, T.; Pyne, S.G. Synthetic and spectroscopic studies on the structures of uniflorines A and B: Structural revision to 1,2,6,7-tetraydroxy-3-hydroxymethylpyrrolizidine alkaloids. Tetrahedron, 2008, 64, 4868-4879.

Karanjule, N.S.; Markard, S.D.; Bhavale, D.D. Synthesis of diphényltetrahydroxyindoline alkaloids using ring closing metathesis: Attempts to find the correct structure of uniflorine A. J. Org. Chem., 2006, 71, 6273-6276.

Zhao, Z.; Song, L.; Mariano, P.S. A concise sequential photochemical-maturation of lysosomal α-galactosidase A in Fabry lymphoblasts by an epimer. J. Org. Chem., 2011, 76, 10786-10800.

Bell, A.A.; Pickering, L.; Watson, A.A.; Nash, R.J.; Griffiths, R.C.; Jones, N.M.G.; Fleet, G.W.J. 2-Hydroxycastanospermine (dihydroxy-1-swainosine) from ootonolactones: Inhibition of naringine (L-rhamnososid). Tetrahedron Lett., 1996, 37, 8561-8564.

Ameijde, J.V.; Horne, G.; Wormald, M.R.; Dwek, R.A.; Nash, R.J.; Jones, P.W.; Evinson, E.L.; Fleet, G.W.J. Isolation and synthesis of glycosidase inhibition profile of 3-epi-casuarine. Tetrahedron, 2006, 17, 2702-2712.

The Angiosperm Phylogeny Group. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG III. The Angiosperm Phylogeny Group. J. Syst. Evol., 2009, 47, 105-121.

Nash, R.; Watson, A.A.; Asano, N. Alkaloids: Chemical and biological Perspectives. Elsevier: Oxford, 1996, Vol. 11.

Thurber-Pelham, R.H. Suspected Scilla non-scripta (bluebell) poisoning in cattle. Vet. Rec., 1967, 80, 709-710.

Watson, A.A.; Nash, R.J.; Wormald, M.R.; Harvey, D.J.; Dealler, S.; Lees, E.; Asano, N.; Kizu, H.; Kato, A.; Griffiths, R.C.; Caines, A.J.; Fleet, G.W.J. Glycosidase-inhibiting pyrroline alkaloids from Hyacinthoides non-scripta. Phytochemistry, 1997, 46, 255-259.

Kato, A.; Adachi, I.; Miyuchii, M.; Ikeda, K.; Komae, T.; Kizu, H.; Kameda, Y.; Watson, A.A.; Nash, R.J.; Wormald, M.R.; Fleet, G.W.J. Asano, N. Polyhydroxylypyrrolizidine and pyrrolizidine alkaloids from Hyacinthoides non-scripta and Scilla campanulata. Carbohydry. Res., 1999, 316, 95-103.

Asano, N.; Kuroi, H.; Ikeda, K.; Kizu, H.; Kameda, Y.; Kato, A.; Adachi, I.; Watson, A.A.; Nash, R.J.; Fleet, G.W.J. New polyhydroxylypyrrolizidine alkaloids from Muscari armeniacum. Tetrahedron, 2000, 56, 1-8.

Mehta, A.; Zitzmann, N.; Rudder, P.M.; Block, T.M.; Dwek, R.A. α-Glucosidase inhibitors as potential broad-based anti-viral agents. FEBS Lett., 1998, 430, 17-22.

Watson, A.A.; Fleet, G.W.J.; Asano, N.; Molyneux, R.J.; Nash, R.J. Polyhydroxy alkaloids - natural occurrence and therapeutic applications. Pharmacol. Rev., 2001, 53, 63-140.

Platt, F.M.; Neises, G.R.; Reinkensmeier, G.; Townsend, M.J.; Perry, V.H.; Robertson, J.; Stevens, K. Pyrrolizidine alkaloids: Occurrence, biology, and chemical synthesis. Academic Press: San Diego, 2004, pp. 1-314.

Fellows, L.E.; Evans, S.V.; Nash, R.J.; Bell, E.A. Polyhydroxy plant alkaloids as glycosidase inhibitors and their possible ecological role. ACS Symp. Ser., 1986, 296, 72-78.
[77] Appel, R. Tertiary phosphane/tetrachloromethane, a versatile reagent for chlorination, dehydration, and P-N linkage. *Angew. Chem. Int. Ed. Engl.*, 1975, 14, 801-811.

[78] Jasinski, M.; Moreno-Clavijo, E.; Reissig, H.-U. Synthesis of a series of enantiopure polyhydroxylated bicyclic N-heterocycles from an L-erythrose-derived nitroxyallenes. *Eur. J. Org. Chem.*, 2014, 2, 442-454.