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Synthesis of leptosin, a glycoside isolated from mānuka honey

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ABSTRACT

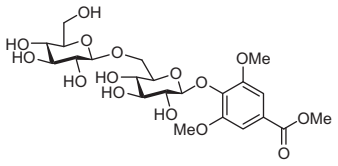
The first synthesis of leptosin, a novel glycoside isolated from mānuka honey is described. Utilising an acetyl protecting group strategy the glycoside was obtained with excellent anomeric selectivity by deploying a Schmidt glycosylation as a key step.

For thousands of years honey has been used to treat a disparate range of ailments.[1] In the past decade, however, the medical use of honey has undergone somewhat of a renaissance.[2] In particular, mānuka honey (*Leptospermum scoparium* or *Leptospermum polygalifolium*) shows strong antibacterial properties, which had led to a growing demand for honey-impregnated wound care products and other medicinal products.[3] Unlike other honeys, mānuka honey contains methyl glyoxal, which has been identified as one of the major contributors to its biological activity.[4]

Furthermore, other unique compounds, such as certain phenols may also play a role in its biological profile.[5] These phenolic compounds may in part be responsible for the anti-inflammatory bioactivity, aiding the wound-healing process.[6] As a result, these compounds are becoming increasingly recognised for their potential role in treating microbial infections and reducing inflammation,[5c,7] although their exact mode of action is poorly understood.[4]

The increase in demand for medical mānuka honey has driven prices up and at present there are few quality assurances for those producing and distributing honey. Consequently, the industry has become a target for fraudulent marketing and counterfeiting. As such, there is significant interest in the development of a set of chemical markers specific to mānuka honey, which can be used to determine the identity and purity via HPLC or mass spectrometry analysis.[8]

Recently, Kato and co-workers reported the isolation of leptosin (**1**), consisting of methyl syringate and the disaccharide gentiobiose connected via a β-glycosidic linkage.[9] After testing a variety of different honeys, they found that leptosin (**1**) is only present in mānuka honey,[9] suggesting that it may be a suitable chemical marker for mānuka honey 'fingerprinting'. We therefore focused on the synthesis of leptosin (**1**) to ensure ready access to this compound as an internal standard for honey analysis as the isolation of the natural material is low yielding and inefficient[9] (Fig. 1).



leptosin, **1**

Figure 1. Methyl syringate 4-*O*-β-D-gentiobiose (leptosin **1**).

In our initial strategy we decided to synthesise leptosin (**1**) via Koenigs–Knorr glycosylation of phenol **3** with gentiobiosyl bromide (**4**), followed by a Zemplén deacetylation (Scheme 1). In turn it was anticipated that Koenigs–Knorr glycosylation of bromide **5** and primary alcohol **6**, both derived from glucose, would provide gentiobiosyl bromide (**4**). Although gentiobiose is commercially available, it is expensive for use in synthesis (NZ$647 per gram from Sigma Aldrich).

Starting from D-glucose (**7**), the glycosyl bromide **5** was prepared in a one-pot procedure using acetic anhydride and perchloric acid in glacial acetic acid followed by the addition of acetyl bromide and methanol (Scheme 2). The desired 2,3,4,6-tetra-*O*-acetylglucosyl bromide **5** was isolated in excellent yield.[10] Glycosyl

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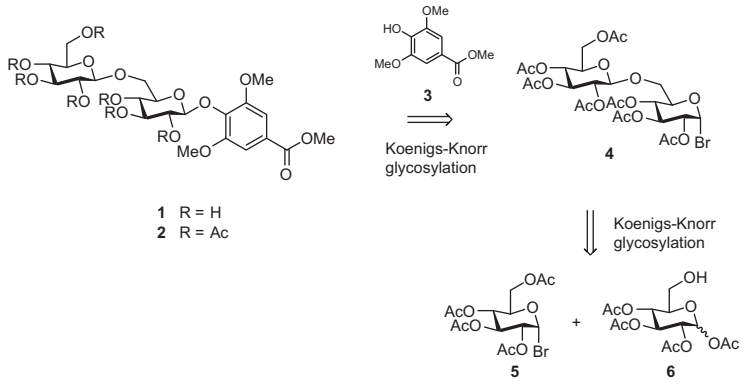
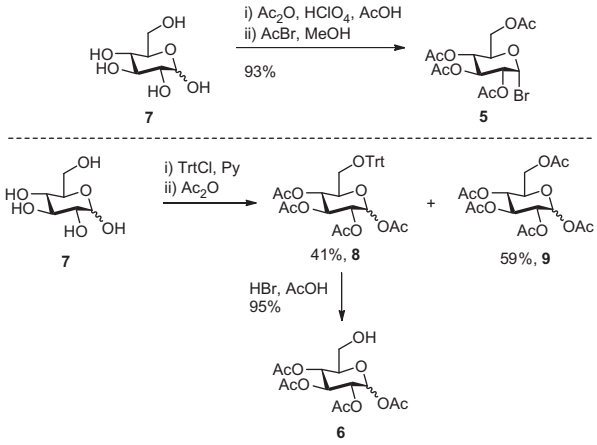


Scheme 1. Retrosynthesis of leptosin (**1**).



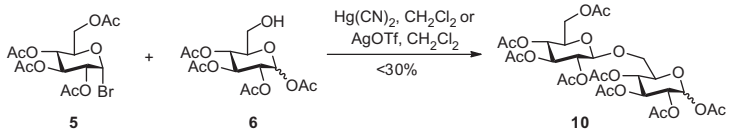
Scheme 2. Synthesis of glycosyl donor **5** and acceptor **6**.

acceptor **6** was synthesised in two steps, also starting from D-glucose (**7**). Firstly, the primary alcohol was protected as a trityl ether, followed by peracetylation to afford a mixture of the desired 1,2,3,4-tetra-*O*-acetyl-6-*O*-trityl-glucose **8** and 1,2,3,4,6-penta-*O*-acetylglucose **9** in 41% and 59% yields, respectively. Cleavage of the primary trityl ether was accomplished by reaction with hydrogen bromide in glacial acetic acid to afford the glycosyl acceptor **6** in 95% yield after flash chromatography[11] (Scheme 2).

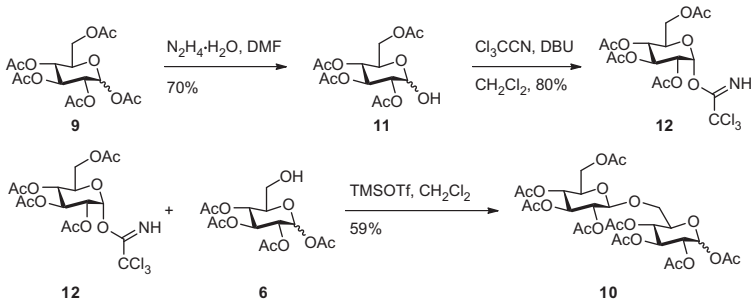
Attention next focused on the synthesis of the disaccharide **10** via a Koenigs–Knorr glycosylation[12] of glycosyl donor **5** with glycosyl acceptor **6** (Scheme 3). Silver(I) triflate was used as the promotor, following the literature precedent for disaccharide synthesis,[13] but the desired product **10** could only be isolated in less than 30% yield. Switching to mercuric(II) cyanide as a promoter applying the Helferich conditions[14] did not improve the yield of the envisaged disaccharide **10**. Significant hydrolysis of the glycosyl donor **5** was observed together with other uncharacterised side products.

Given that attempts to use anomeric bromide **5** as the glycosyl donor to access gentobiose derivative **10** resulted in limited success, our focus turned to the use of a Schmidt glycosylation, which is known to be a robust and reliable glycosylation method.[15] Towards this end, peracetylated glucose **9** was selectively deprotected at the anomeric position using hydrazine hydrate to give the alcohol **11** in 70% yield (Scheme 4).[16] Alcohol **11** was then transformed into trichloroacetimidate **12** under standard conditions (Cl_3CCN, cat. DBU), selectively affording the α-anomer **12** in 80% yield. Pleasingly, activation of trichloroacetimidate **12** with trimethylsilyl triflate in the presence of glycosyl acceptor **6** afforded gentiobiose derivative **10** in 59% yield after flash chromatography.

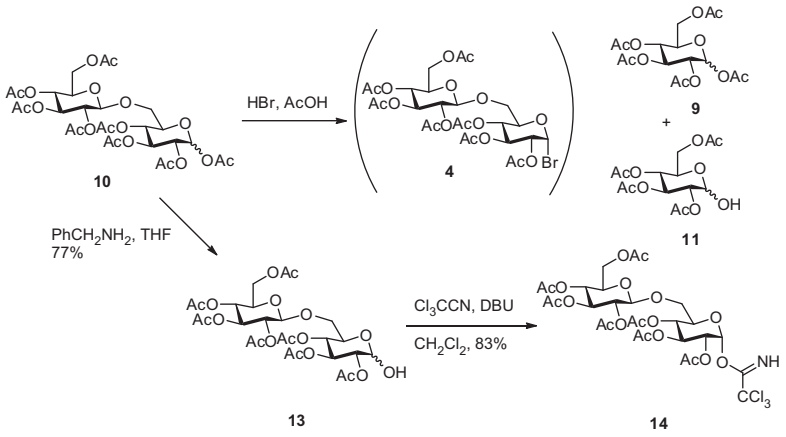
With the successful preparation of peracetylated gentiobiose **10** in hand, our attention turned to the synthesis of a suitable donor



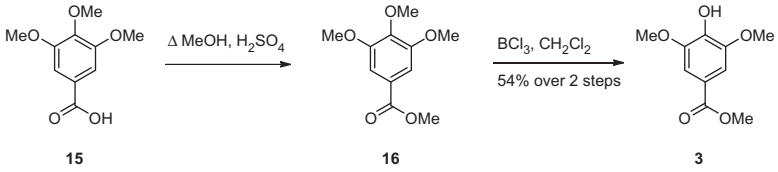
Scheme 3. Glycosylation conditions to form gentiobiose **10**.



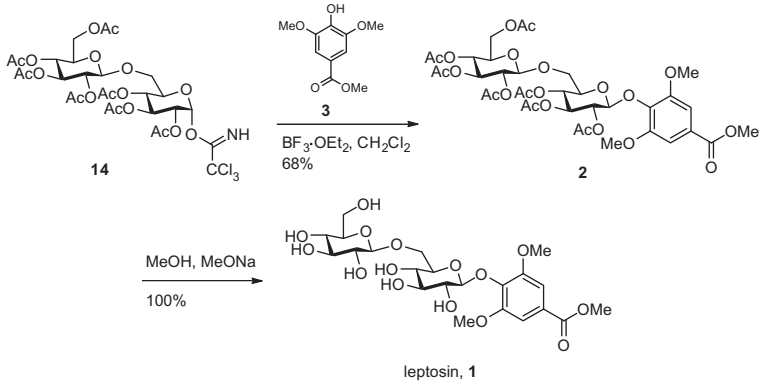
Scheme 4. Revised synthesis of gentiobiose **10**.



Scheme 5. Synthesis of the Schmidt donor **14**.



Scheme 6. Synthesis of phenol **3**.



Scheme 7. Final steps in the synthesis of leptosin (**1**).

for the subsequent glycosylation. Generation of the anomeric bromide **4** by reaction of **10** with hydrogen bromide in glacial acetic acid proved challenging, and to our surprise, the reaction resulted in cleavage of the glycosidic bond, producing peracetyl- and tetraacetyl glucose (Scheme 5). We therefore decided to adopt the Schmidt protocol cleaving the anomeric acetate **10** by reaction with hydrazine hydrate as described earlier to afford anomeric alcohol **13**. Unfortunately, cleavage of the acetate using hydrazine hydrate was unselective and partial degradation was also observed. Use of milder conditions, namely benzylamine in THF, gave good selectivity resulting in the formation of the anomeric alcohol **13**, in 77% yield. Alcohol **13** was then converted into trichloroacetimidate **14** by reaction with trichloroacetonitrile and a catalytic amount of DBU to give **14** in 83% yield.

Attention next turned to the glycosylation of **14** with phenol **3**, thus requiring access to the glycosyl acceptor **3**. Phenol **3** was synthesised from 3,4,5-trimethoxybenzoic acid **15** in two steps (Scheme 6). Initially, methyl ester **16** was formed under acidic conditions followed by the selective demethylation of the *para*-methyl ether using boron trichloride in 54% yield over two steps.[17]

The key step in the synthesis of leptosin is the glycosylation reaction of phenol **3** with glycosyl donor **14**. To accomplish this critical glycosylation we utilised boron trifluoride diethyl etherate, following the literature precedent that suggested it as a superior promoter for glycosylations with phenol acceptors.[18] Gratifyingly, we found that these conditions afforded peracetylated leptosin **2** as a single anomer in 68% yield (Scheme 7).

Finally, Zemplén deacetylation[19] was carried out in methanolic sodium methoxide to afford leptosin (**1**) in quantitative yield as a colourless solid. All spectroscopic and analytical data of the synthetic material were in agreement with that published earlier by Kato and co-workers for the natural material.[9]

In summary, we have developed a quick and reliable synthesis of leptosin (**1**), a unique glycoside present only in mānuka honey. Key steps are the Schmidt glycosylation between the anomeric trichloroacetimidate **12** and primary alcohol **6**, as well as between

donor **14** and phenol **3** to construct the glycosidic linkages. The overall yield is 14% over eight steps for the longest linear sequence starting from D-glucose. This synthetic route will enable access to leptosin (**1**) for use as an internal standard to identify mānuka honey by HPLC analysis as well as mass spectrometry.

Acknowledgments

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Supplementary data

Supplementary data (experimental procedures and ^{1}H and ^{13}C NMR spectra for compounds **1**, **2**, **10**, **13**, and **14**) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2013.10.042.

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