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Synthesis and biological evaluation of novel biotin–iminoalditol conjugates

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ABSTRACT

Biotin–iminosugar conjugates of different configuration such as D-*gluco*, D-*galacto*, L-*ido* as well as a furanoid representative in the D-*manno* configuration have been synthesised and exhibit powerful inhibition of β-glucosidase from *Agrobacterium* sp. with K_i values in the range of the respective parent compounds. Such molecular probes have potential for activity-based protein profiling taking advantage of the biotin–(strept)avidin interaction.

Iminosugars including iminoalditols and related bicyclic alkaloids are the most prominent family of low molecular weight competitive glycosidase inhibitors.[1] Paradigmatic examples such as compounds **1**, **2**, **3** and **4** (Fig. 1) and quite a few of their derivatives have found important roles as diagnostic compounds such as in the investigation of glycoprotein-trimming glycosidases[2] or as pharmaceutical substances for example in the treatment of diabetes type II symptoms[3] as well as hereditary enzyme deficiency diseases.[4] Other significant biological activities associated with their glycosidase inhibitory properties are anti-viral, anti-cancer and anti-metastatic, anti-infective as well as insect anti-feedant and plant growth regulatory effects.[5]

Recently, we have found that various fluorescently labelled iminosugar derivatives such as compounds **1a**, **2a**, **2b** as well as **4a** are strikingly powerful glycosidase inhibitors exceeding the parent compounds' activities by up to two orders of magnitude (Fig. 2,[6,7]).

Such labelled inhibitors were deemed to be highly useful diagnostic tools for activity-based high-throughput analysis as well as gel staining. For example, we designed and constructed an iminoalditol chip with three typical glycosidase-inhibiting iminoalditols attached to a polyamine surface displayed on a silicon chip.[7] Exposure to representative β-glucosidases revealed selective binding events reflecting the different structural features of the inhibitors, providing a proof-of-concept for the successful exploitation of microarrays of typical reversible glycosidase inhibitors of the iminosugar family.

Activity-based protein profiling (ABPP) of exo- as well as endo-glycosidases has been shown to be a versatile tool for the investigation of their assignment of structures and their functions.[8] A typical probe for ABPP represents a mechanism-based inactivator specific to the active site of the enzyme under investigation conjugated via a noncleavable or cleavable linker to biotin which allows for affinity chromatography or fluorescence spectroscopy.[9] In general, the biotin–(strept)avidin interaction has been proven to be an indispensable tool for biorecognition in the context of diagnostics, biotechnology and nanotechnology.[10]

We have prepared three biotinylated representatives of six-membered ring iminosugars with the D-*gluco*, D-*galacto* as well as L-*ido* configuration **10**, **11** and **15**. In addition, we have prepared the biotinylated derivative **6** of the five membered glycosidase inhibitor 2,5-dideoxy-2,5-imino-D-mannitol (**2**).

Biotin–iminosugar conjugate **6** (Scheme 1) was obtained by coupling of 1-amino-1,2,5-trideoxy-2,5-imino-D-mannitol (**5**)[11] to biotin employing *O*-benzotriazole-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluoro-phosphate (HBTU) as the coupling reagent in DMF and in the presence of triethylamine in 50% yield.

Compounds **10** as well as **11** (Scheme 2) were obtained by coupling of aminoethylaminobiotin **9** to *N*-(carboxypentyl)-1-deoxynojirimycin **7**[12] and *N*-(carboxypentyl)-1-deoxygalactonojirimycin **8**, respectively, under the same coupling conditions and yields of 58% and 50%. Mono-biotinyl-1,2-diaminoethane (**9**) was prepared by coupling of biotin to one terminus of ethylendiamine using HBTU as the coupling reagent.

For the preparation of L-*ido* iminosugar **15** (Scheme 3) we started from 1-deoxy-L-idonojirimycin **12**.[13] Reductive amination

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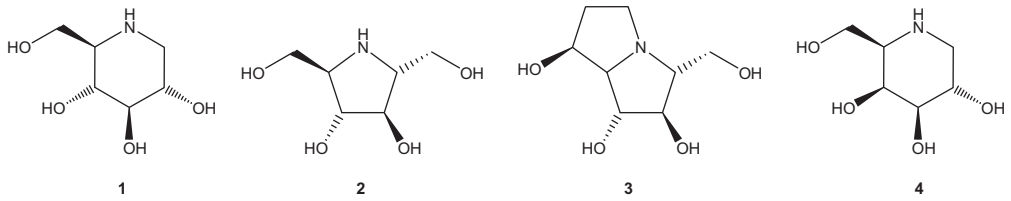


Figure 1. Structures of iminosugars **1**–**4**.

with adipic acid methyl ester hemicarbaldehyde **13** in methanol using Pd/C as the catalyst led to *N*-(methoxycarbonylpentyl)-1-deoxy-L-ido-nojirimycin **14** in a yield of 51%. Saponification of the ester in 1,6-dioxane/water gave the terminal carboxylic acid which again was coupled to biotin derivative **9** using HBTU in DMF and triethylamine yielding the respective biotinylated L-*ido* iminosugar **15** in 43% yield.

Inhibition constants (K_i values, Table 1) of biotinylated compounds **6**, **10**, **11** and **15** for the *Agrobacterium* sp. β-glucosidase were found in the range of those of the corresponding parent iminosugars. In line with previous observations with N-modified compounds in the D-*galacto* series, compound **11** exhibits nicely improved selectivity for the β-selective enzymes probed.[14] Thus, these probes are clearly recognised by the enzyme and may have great potential for various applications including glycosidase profiling.

In conclusion we have synthesised the first biotin–iminosugar conjugates and have shown that these N-modified structures act as glycosidase inhibitors. The synthetic strategy can be applied to a wide range of iminosugars and allows for easy modification of

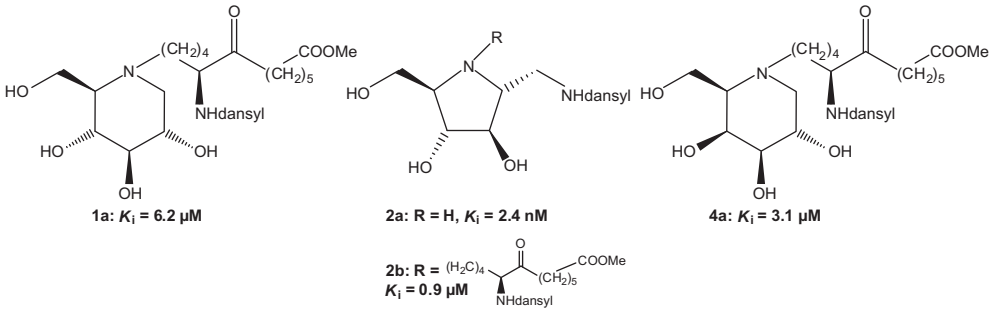
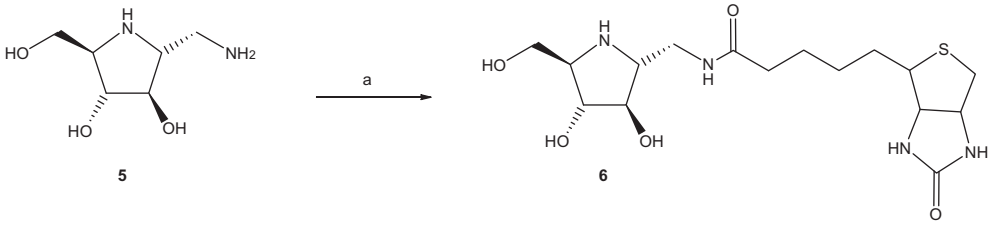
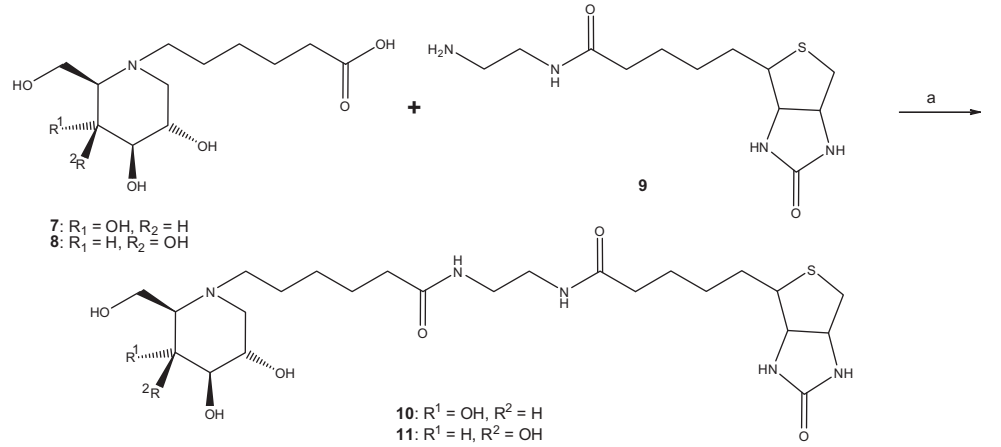


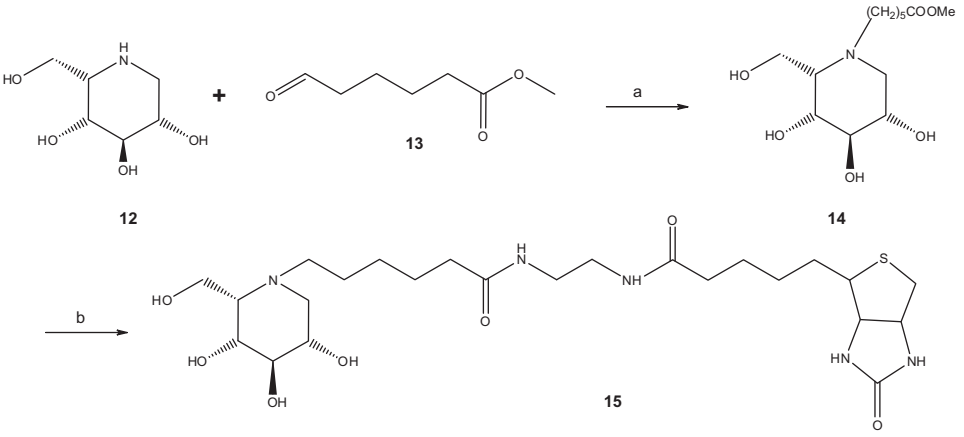
Figure 2. Structure and K_i values against *Agrobacterium* sp. β-glycosidase of paradigmatic example of fluorescently labelled iminosugars.



Scheme 1. Synthesis of compound **6**. Reagents and conditions: (a) Bitoin, HBTU, Et_3N, DMF, rt, 5 h, 50%.



Scheme 2. Synthesis of biotin–iminosugar conjugates **10** and **11**. Reagents and conditions: (a) HBTU, Et_3N, DMF, rt, 5 h, 58% (**10**) and 50% (**11**).



Scheme 3. Synthesis of biotinylated iminosugar **15**. Reagents and conditions: (a) Pd/C, H_2, MeOH, rt, 10 h, 51%; (b) (i) NaOMe, water/dioxane, rt, 12 h; (ii) compound **9**, HBTU, Et_3N, DMF, rt, 12 h, 43%.

Table 1
Inhibitory activities (K_i values in μM) of biotinylated iminosugars **6**, **10**, **11** as well as **15** with β-glycosidase from *Agrobacterium* sp. (β-glu/gal Abg), *E. coli* (β-gal *E. coli*) as well as with the α-galactosidase from green coffee beans (α-gal GCB)

Compound	K_i (μM) β-glu/gal Abg	K_i (μM) β-gal *E. coli*	K_i (μM) α-gal GCB
6 (**2**)	0.5 (0.2)	nd	nd
10 (**1**)	60 (12)	nd	nd
11 (**4**)	11 (100)	2.3 (13)	1.1 (0.013)
15 (**12**)	30 (26)	nd	nd

nd—not determined.

the linker length. Such biotinylated glycosidase inhibitor conjugates are envisaged as helpful tools for activity-based protein profiling of glycoside hydrolases. Additionally, the biotin covalently bound to the enzyme inhibitor allows for detection or purification of proteins by affinity binding to (strept)avidin-carrying molecules or surfaces.

Acknowledgements

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Supplementary data

Supplementary data (general methods, biological data as well as experimental and analytical details for the synthesised compounds) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.05.084.

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