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Concise synthesis of C-1-cyano-iminosugars via a new Staudinger/aza Wittig/Strecker multicomponent reaction strategy

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ABSTRACT

A new Staudinger/aza Wittig/Strecker multicomponent reaction sequence to C-1-cyano iminoalditols has been developed. When applied to 5-azidodeoxy-D-xylose and -D-glucose as substrates the method leads smoothly in good yield and with excellent stereoselectivity to respectively, 1,5-dideoxy-1,5-imino-D-idurono nitrile and 2,6-didesoxy-2,6-imino-D-*glycero*-D-*ido*-heptononitrile.

Iminosugars, such as compounds **1**–**3** (Fig. 1), are sugar analogous in which the ring oxygen is replaced by trivalent nitrogen. Representatives of this substance class are basic and well known as powerful and competitive inhibitors of glycoside processing enzymes. Several iminosugars also exhibit anti-microbial as well as anti-cancer properties while some have been reported as immune system stimulating agents and others as pharmacological chaperones (PC).[1]

This latter activity stems from the ability of certain iminosugars to stabilize the folding of mutant lysosomal enzymes and thereby prevent their cellular clearance. As a consequence such compounds have shown promise for the treatment of various lysosomal storage diseases (LSD).[2] Application as therapeutics for the treatment of abnormal glycosphingolipid metabolism as well as for diabetes type II, through inhibition of membrane bound β-glucosidase II has also been proposed.[3] Recently, carbohydrates have been implicated to play a role in Alzheimer's and Parkinson diseases,[4] further adding to the interest of iminosugars as potential drug candidates.

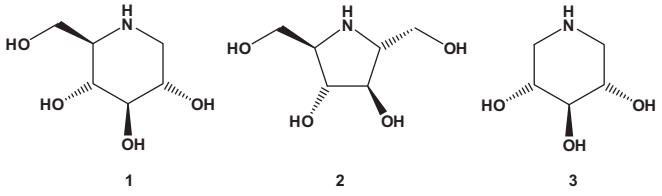

Figure 1. Structures of iminoalditols **1**–**3**.

A key concern in the use of iminoalditols as therapeutic agents is that they very often interact not only with their intended glycosyl hydrolase target, but also with others. Lack of specificity sometimes leads to undesired side-effects and a number of strategies have been proposed to modify the parent iminosugar skeleton so as to address this problem. These include changing the substitution pattern or configurations of the iminosugar scaffold as well as introducing substitutions such as *C*-glycosyl functions. The hope is that such changes might lead to compounds that additionally interact with a particular enzymatic aglycone binding site thereby enhancing its overall potency and selectivity.[5]

For example, Martin and co-workers synthesised and biologically evaluated 1,5-dideoxy-1,5-imino-D-xylitol (DIX) derivative **4** (Fig. 2), which carries an α-*C*-glycosidically linked alkyl chain at

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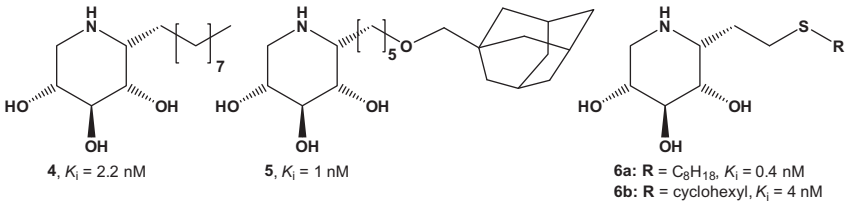


Figure 2. Structures of iminoalditols **4**, **5** and **6**.

position C-1 and found this to enhance inhibitory potency and selectivity towards the target human lysosomal β-glucocerebrosidase (GCase) as well as their PC activity.[6] Parallel observations were made by Overkleeft and coworkers when investigating the (1*R*)-1-*C*-[4-(adamant-1-yl-methoxy)butyl]-DIX **5**.[3] Recently, Withers and co-workers synthesised a library of iminoxylitols featuring α-1-*C*-thioalkyl or -thiophenyl substituents (e.g., compounds **6a** and **6b**), and found several to show very good K_i values as well as PC activities with GCase.[7]

We have been interested in piperidine as well as pyrrolidine type iminosugar analogues for some time and especially in the tuning of their biological activities towards a particular function. Thus the introduction of diverse skeletal decorations, such as reporter groups, ionisable groups, aromatic systems and perfluoroalkyl groups, has led to our discovering compounds with interesting activities as PCs towards various lysosomal enzyme mutants. This endeavour has required the development of efficient routes for the synthesis of a prerequisite iminosugar skeleton as well as its subsequent decoration.[8]

Herein we describe the development of a new multicomponent reaction (MCR) strategy for the synthesis of *C*-glycoside iminosugars. Past methods to iminosugar-*C*-glycosides are numerous[9] but few exploit MCR sequences although these number amongst the most powerful in organic chemistry.[10] The Staudinger/aza-Wittig/Strecker (SAWS) reaction sequence described herein allows the efficient preparation of 1-*C*-cyano-iminosugars. A key step in our approach is the cyanide ion trapping of an iminium ion intermediate generated from an appropriate azidodeoxy carbohydrate substrate. The Staudinger/aza-Wittig/Ugi 3 component sequence, introduced by Overkleeft also implicates an iminium ion intermediate.[11] Further, the 1-*C*-cyano-iminosugars obtained through our SAWS sequence prove to be versatile building blocks and amenable to convenient elaboration. This has allowed the construction of functionalised iminoalditols customized for various applications in a straightforward manner.

In the proposed strategy (Scheme 1), a Staudinger reaction would first be initiated through the reaction of a trialkyl phosphine, with an azidodeoxy aldose **I** leading to the formation of the corresponding phosphazene **II**. This species then reacts intramolecularly with the aldehyde function to undergo an aza-Wittig reaction giving the corresponding cyclic imine intermediate **III**, which is susceptible to trapping by cyanide ion. This last Strecker reaction results in the incorporation of a new stereocenter at the indicated position and gives the target 1-*C*-cyano-iminosugar building block **IV**.

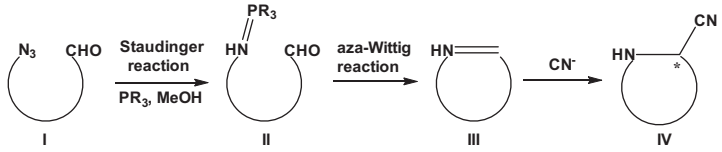
The feasibility of the new SAWS reaction sequence was first tested on the substrate 5-azido-5-deoxy-D-xylose (**6**, Scheme 2), easily accessible from D-xylose.[12] Reaction of **6** with trimethyl phosphine (PMe_3) in methanol followed by addition of sodium cyanide gave the expected 1-*C*-cyano-imino DIX **9** in an excellent overall yield of 98%. The postulated intermediates **7** and **8** were not isolated or identified. Per-O-acetylation of the crude 1-*C*-cyano DIX **9** analogue allowed convenient isolation via its corresponding acetate derivative **10**.

The configurations at the newly generated chiral centers in compounds **9** and **10** were determined by NMR analysis. The coupling constants between H-2 and H-3 of 5.6 and 5.7 Hz, respectively indicate that the cyanide function has been introduced from the α-side and is in a *cis*-relationship to the hydroxyl group at position C-2. This assignment has been confirmed through an X-ray structure of compound **9**.[13]

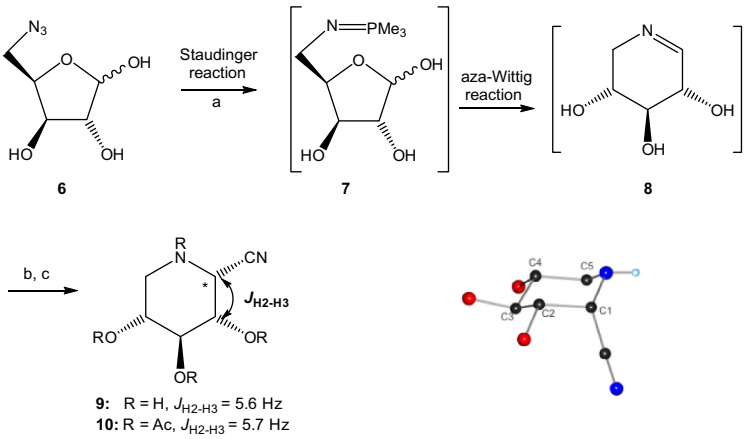
These MCR conditions were then applied to the alternate substrate, 5-azido-5-deoxy-D-glucose **11** (Scheme 3), synthesised from D-glucuronolactone as described earlier.[14] As before, the sequence saw the cyanide group being introduced stereoselectively to give the expected 1-*C*-cyano-1-deoxynojirimycin **12** as a single stereoisomer in 65% yield. Again per-O-acetylation of **12** allowed its convenient isolation as its corresponding acetate derivative **13**. Assignment of the stereochemistry at C-1 was possible from an analysis of NMR data ($J_{H2,H3}$ = 5.7 Hz in compound **12** and $J_{H2,H3}$ = 5.2 Hz in compound **13**) and proved for compound **13** by its X-ray structure.[15] These data confirm that cyanide trapping of the intermediate iminium species occurs on the same face as the hydroxyl group at position C-2 to give **12**.

The 1-*C*-cyano iminosugar products have the potential to serve as diversely functionalisable building blocks: through reduction of the nitrile group and subsequent chemo- and regioselective alkylation, acylation or sulfonation reactions of the primary amine function thereby revealed.[17] For example, reduction of the cyanide group in compound **9** occurs smoothly employing platinum oxide in MeOH/HOAc under a hydrogen pressure of 5 bar to furnish 1-*C*-aminomethyl-DIX **14**, which underwent clean N-dansylation with dansyl chloride in methanol and triethylamine to give fluorescent DIX derivative **15** (Scheme 4). This compound was found to adopt two different conformations, depending on the pH-value, as has been observed previously for other *C*-glycosyl iminoxylitols:[6,8] Under basic conditions this compound prefers a $^5C_2$ conformation whereas a $^2C_5$ conformation, in which the hydroxyl groups are oriented in an all axial position, is adopted under acidic conditions.

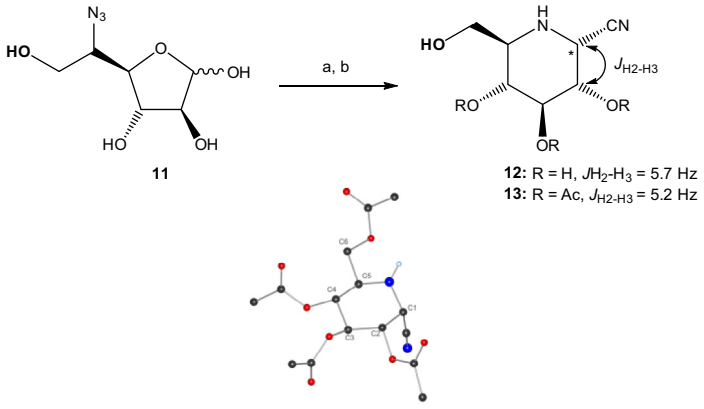
The kinetic evaluation of compounds **9**, **15** and **12** as inhibitors of two well-characterised β-glucosidases is summarised in Table 1. Consistent with previous studies,[6–8] the two D-*xylo*-configured iminosugars **9** and **15** are good inhibitors of human GCase with K_i values of 34 μM and 7.5 nM, respectively, with the lipophilic dansyl group at C-2 clearly contributing greatly to the elevated



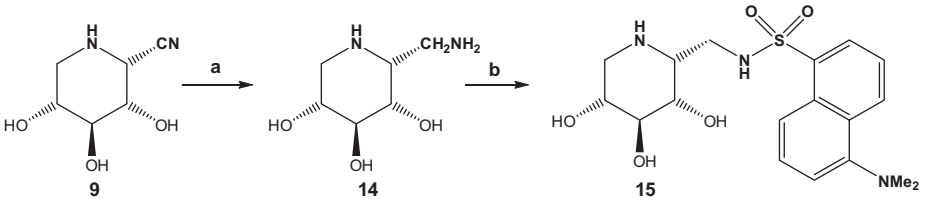
Scheme 1. Staudinger/aza-Wittig/Strecker reaction sequence.



Scheme 2. Synthesis of 1-*C*-cyano-DIX **9** and assignment of the new chiral centre at position C-2 by ^{1}H NMR and X-ray structure data. (a) PMe_3, MeOH, (b) NaCN, (c) Ac_2O, pyr.



Scheme 3. Synthesis of 1-*C*-cyano-1-deoxynojirimycin derivative **12** and X-ray structure of compound **13**,[16] (a) PMe_3, NaCN, MeOH, (b) Ac_2O, pyr.



Scheme 4. Synthesis of dansylated DIX derivative **15**. (a) PtO_2, H_2, MeOH, 5 bar, (b) dansylchloride, MeOH, Et_3N.

Table 1
Inhibition constants (K_i, μM) of compounds **1**, **3**, **9**, **12** and **15** with lysosomal β-glucocerebrosidase (GCase) and β-glucosidase *Agrobacterium* sp. (Abg)

Compound	K_i (μM) GCase	K_i (μM) Abg
1	79[6]	12[18]
3	1.3[6]	50[12]
9	34 (±12)	220 (±19)
12	716 (±69)	172 (±29)
15	0.0075 (±0.6)	36 (±4)

affinity of **15**. The corresponding D-*gluco* configured 1-*C*-cyano precursor **12** is, in contrast, a more modest inhibitor with a K_i value of 716 μM for the human GCase and 172 μM for β-glucosidase from *Agrobacterium* sp. However, it would serve as a useful platform for further elaboration: Reduction of the cyanide function followed by introduction of lipophilic substituents is expected to lead to analogues of **12** with increased inhibitory activity.

In conclusion, a Staudinger/aza-Wittig/Strecker (SAWS) reaction sequence has been developed as a new route to C-1-cyano iminosugar analogues. Its successful application to two substrates, 5-azido-5-deoxy-D-xylose (**6**) and 6-azido-6-deoxy-D-glucose (**11**), has provided the corresponding 1-*C*-cyano-1,5-iminosugar-alditols, **9** and **12**, respectively in good yields and with complete stereoselectivity. The configurations of the newly introduced chiral centers in **9** and **12** have been supported by NMR spectroscopic data and confirmed by X-ray structure data. Transformation of the 1-*C*-cyano analogue **9** is shown to be straightforward and gave the dansylated iminosugar derivative **15**. The latter analogue proves to be a nanomolar inhibitor of human lysosomal GCase. This newly developed SAWS strategy has considerable scope for the development of focused libraries of iminosugar-*C*-glycosides with potential not only as potent inhibitors of glycosidases but also as PCs for the treatment of LSDs such as Gaucher disease. Implementation of this MCR route to alternative azidodeoxy sugar substrates

is expected to furnish a variety of alternative *C*-cyano analogues, and these, when subjected to potential follow-up chemistries, promise to provide a number of very interesting focussed compound libraries. We are persuaded that methods through which the convenient synthesis of large numbers of compounds is possible will accelerate the development of leads optimized for a selected biological function. The investigation of such approaches is currently in progress in our laboratories.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2014.03.069.

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