Biosynthetic short activation of the 2,3,6-trideoxysugar l-rhodinose

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The successful incorporation of non-activated [2,3-$^2H_2$]-L-rhodinose 4b into landomycin A 7 proves the existence of a short activation pathway for L-rhodinose 4; this result provides evidence of a general saving pathway for deoxysugar building blocks and their biosynthetic intermediates, respectively, which become accidentally deactivated during the biosynthetic deoxygenation cascades.

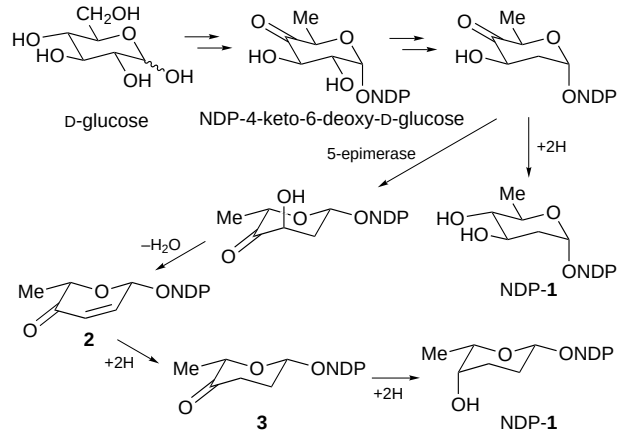
Deoxysugars, for example l-rhodinose **4** and d-olivose **1**, occur as structural moieties of many bioactive natural products and often play an important role in their mechanism of action, in particular for the DNA interaction of anti-cancer drugs.[1] For instance, the cytostatic activity of the different landomycins,[2] anti-tumour antibiotics of the angucycline group[3] which vary only in their oligosaccharide moieties, is directly dependent on the length of the phenolglycosidically-linked deoxyoligosaccharide chains.[2c] Landomycin A **7**, the most active anti-cancer compound of the landomycin family, exhibits the longest glycan moiety. While we have been investigating the biosynthesis of this hexadeoxysaccharide chain and the pathways leading to its two single deoxysugar building blocks,[2d–f] d-olivose (**1**, 2,6-dideoxy-d-*arabino*-hexopyranose) and l-rhodinose (**4**, 2,3,6-trideoxy-l-*threo*-hexopyranose), we have also been working on the development of synthetic methods in this context.

For the biosynthetic formation of deoxyhexoses it is generally accepted that the deoxygenation steps occur on nucleoside diphosphate (NDP) intermediates,[4] which are activated substrates for glycosyl transfer. The activation pathway always starts with a hexose-6-phosphate, which is transformed into the activated NDP-sugar by a pyrophosphorylase after its rearrangement into the corresponding hexose-1-phosphate. This activation mechanism occurs prior to all deoxygenation steps, and thus was assumed to exclude the activation of 6-deoxysugars.

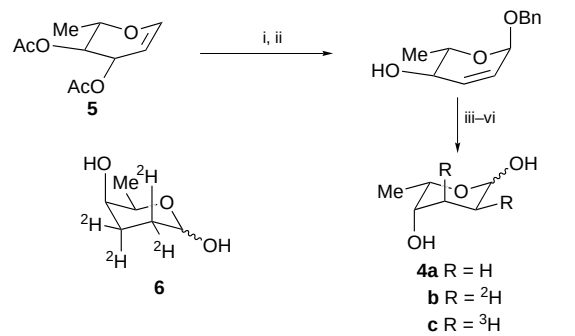
Since diphosphates, and in particular 2-deoxysugar derivatives, are chemically labile,[5] there is a considerably high chance that the activated (deoxy)sugar may be 'deactivated' through hydrolysis, *e.g.* to the trideoxysugar rhodinose (**4**, see Scheme 1). Thus these sugars would be unable to participate in the final biosynthetic glycosyl transfer reactions, if nature did not provide for the possibility of 'recycling', best achieved in a 'short activation pathway'. Such a second activation pathway was first recognized for l-fucose,[6] a 6-deoxysugar, which normally arises as an activated guanosyldiphosphate (GDP)-derivative from GDP-d-mannose.

Feeding experiments, in which synthetically prepared (Scheme 2)[7] unlabelled l-rhodinose **4a** was added to a growing culture of the landomycin A **7** producer *Streptomyces cyanogenus* (strain S-136, DSM 5087) showed that l-rhodinose **4a** was consumed within 12 h (HPLC, RI-detector control), and therefore seemed to be transported into the cells. Furthermore, experiments with radiolabelled [2,3-$^3H_2$]-l-rhodinose **4c** analogously synthesized[7] using $^3H_2$ (prepared[8] from NaB^3H_4 and ^3H_2O) showed incorporation into **7**. However, quenching effects interfered with the scintillation counting and therefore only low specific incorporation was indicated. In addition, it remained unproven whether the radiolabel was exclusively located in the rhodinose moieties of **7**. Thus, the final proof of a successful incorporation of non-activated l-rhodinose into the l-rhodinose moeties of landomycin A (**7**, Scheme 3) was obtained from feeding [2*S*,3*R*-$^2H_2$]-l-rhodinose **4b** (synthesized[7] with $^2H_2$; Scheme 2). The ^2H NMR spectrum clearly showed ^2H enriched signals exclusively for 2C–H_e, 3C–H_a, 2F–H_e and 3F–H_a (Fig. 1). The assignment of all the protons of **7** and its octaacetate, respectively, was achieved *via* long range C,H couplings (COLOC, HMBC).[2a,b] Thus, the ^2H-labelled l-rhodinose **4b** must have been activated through an alternative pathway to the standard deoxygenation pathway (see Scheme 1 for our current hypothesis). This 'short activation pathway' may play the role of a general saving mechanism. A control experiment, in which [2,2,3,3-$^2H_4$]-d-rhodinose **6**, prepared[7] from l-threonine, was fed to *S. cyanogenus*, showed—as expected—no incorporation.

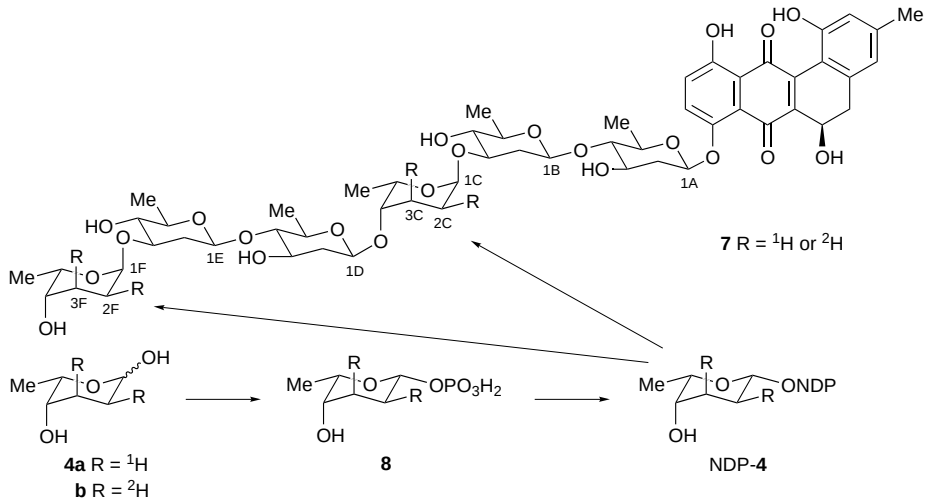
For the short activation it is assumed that l-rhodinose, as was demonstrated for l-fucose,[6] is first converted into the corresponding 1-phosphate **8** by a kinase and then activated into the NDP-derivative (NDP-**4**) through a pyrophosphorylase-mediated coupling reaction (see Scheme 3). That an activation of a 6-deoxyhexose is principally possible has also been indicated



Scheme 1



Scheme 2 *Reagents and conditions*: i, BnOH, $BF_3{\cdot}OEt_2$; ii, MeOH, NEt_3, H_2O; iii, BzOH, DEAD, PPh_3, OH^-; iv, Pd–C, $H_2(^2H_2, ^3H_2)$; v, MeOH, NaOMe; vi, H^+



Scheme 3

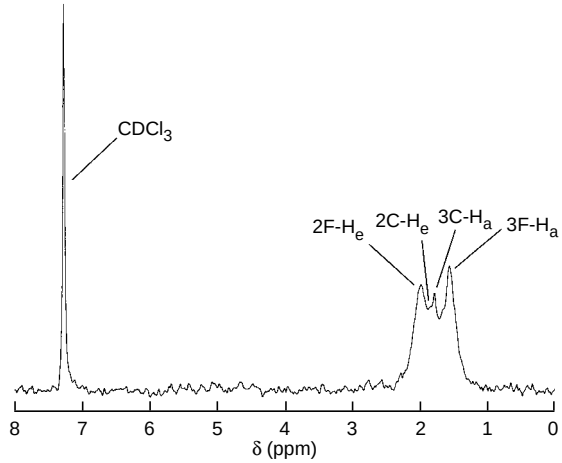


Fig. 1 ^{2}H NMR spectrum of the isolated landomycin A (**7**) after feeding of [2*S*,3*R*-$^2H_2$]-l-rhodinose (**4b**) to growing cultures of *Streptomyces cyanogenus* S-136. ^{2}H signals were assigned based on the ^{1}H signals [ref. 2(*a*), (*b*)].

from experiments in which 6-deoxy-d-glucose derivatives were successfully incorporated into neomycin.[9]

One can assume for organisms which produce deoxysugar-containing natural products that the short activation is feasible for all deactivated 'intermediates' of a certain biosynthetic deoxygenation cascade. Thus, the results described here open up attractive options in the context of chemical and biochemical aspects of deoxysugars. For instance, they pave the way for more convenient biosynthetic investigations on deoxysugar biosynthesis cascades, since it is obviously possible to feed and incorporate isotope labelled deoxysugars, *i.e.* non-activated analogues of the postulated intermediates of a certain deoxygenation pathway. For our investigations, labelled l-aculose or l-cinerulose, whose NDP-derivatives **2** and **3** respectively we assume to be intermediates of NDP-l-rhodinose (NDP-**4**, see Scheme 1), are important candidates to be fed to cultures of the landomycin producer *S. cyanogenus*. As another consequence of our findings, it will be profitable to look for genes of such short activation pathways, since the corresponding enzymes, preferably a kinase plus a pyrophosphorylase, should express a broad substrate flexibility and thus may be able to activate various deoxyhexoses, presumably at least all intermediates of a given deoxygenation cascade. Therefore, these enzymes may serve as powerful tools in enzymatic oligosaccharide syntheses.

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