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# Synthesis of *all-cis* 2,5-imino-2,5-dideoxy-fucitol and its evaluation as a potent fucosidase and galactosidase inhibitor

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ABSTRACT

We here describe a simple and efficient synthetic method for a non-hydrolysable precursor of a GDP-fucose analogue: The synthesis of the racemic aminofuranofucitol **3** from sorbic alcohol by nitroso-Diels–Alder reaction. This '*all-cis*-pyrrolidine', with all substituents occupying a *cis* position, has been determined as a potent inhibitor of α-L-fucosidase and a moderate inhibitor of α- and β-D-galactosidase. The good recognition of this fucose moiety analogue by specific enzymes is thus confirmed. The C-anomeric bond in this particular structure is in the β-position and makes this compound an interesting candidate for further chemical modifications. Influence of the methyl and hydroxymethyl groups on the inhibition potency is discussed.



Among sugars L-fucose is the most hydrophobic one and is, therefore, of particular importance in mammalian oligosaccharides.[1] For instance, the fucose moiety in sialyl Lewis-x tetrasaccharide is essential for the adhesion of leucocytes to the endothelial membrane of blood vessels in an early stage of the inflammatory process of chronical diseases such as arthritis or psoriasis. The fucose donor in the biosynthesis of this tetrasaccharide is the GDP-fucose (Scheme 1). Replacement of the fucose moiety by a non-hydrolysable analogue seems to be an interesting way to influence both cell migration and the inflammatory process.[2–4]

In recent years, much attention has been paid to the synthesis of aminofucose derivatives as precursors. All these compounds have been evaluated against α-L-fucosidase in order to evaluate the specific recognition of this aminosugar part. Derivatives of L-fuconojirimycin **1**[5–10] proved to be very potent nanomolar α-L-fucosidase inhibitors. Also, five-membered ring analogues as 5-amino-L-talitol **2a**,[11] isomers[11a,12] or derivatives[13,14] were found to act as L-fucosidase or fucosyl-transferase[11a,c,d,12] inhibitors (Scheme 2).

The *all-cis* pyrrolidinetriol **3** in fucitol series seems to be a promising β-L-fucose analogue since it possesses the same anomeric configuration of C-β as the GDP-fucose. We had previously attempted to obtain such analogues by alkylation of the nitrone **4** in the L-lyxose series: unfortunately, only the anomeric C-α derivatives as **2b**[14,15] were obtained. They were nevertheless potent fucosidase inhibitors but cannot be considered precursors for the synthesis of GDP-fucose analogues.

We describe here a simple and efficient method for the synthesis of *all-cis* pyrrolidinetriols. For instance, racemic aminofuranofucitol **3** was synthesised using the versatile oxazine chemistry we had developed earlier.[10,16,17] In addition, its inhibitor potency and selectivity against commercial glycosidases is determined and discussed. Chiral synthesis in both the D- or L-series would be possible using chiral chloronitroso-dienophiles derived from D-mannose or D-ribose,[16,18] respectively.

The *all-cis* pyrrolidine L-**3** is already known, isolated from a leguminoseae[19] as well as synthesised enzymatically as a mixture.[20] Its potent inhibitor effect was briefly mentioned.[21]

The racemic *all-cis* pyrrolidine **3** was synthesised from commercial sorbic alcohol **5a** as shown in Scheme 3: Hetero-Diels–Alder reaction with an acyl-nitroso derivative gave mainly the oxazine **6** with *cis*-configuration. Subsequent anti-bishydroxylation of the double bond followed by two inversions of the configuration (first at C(3) by a tandem oxidation/reduction, and second at C(6) by cleavage of the N–O bond and $S_N2$-cyclisation) led to the target pyrrolidine **3**.

The complete synthesis is represented in Scheme 4. The dienic alcohol **5a**, as 80/20 (2*E*,4*E*/2*E*,4*Z*) isomeric mixture, was O-protected with chloromethoxymethyl ether in the presence of a tertiary amine to give the dienyl ether **5b** in good yield (also as 80/20 isomeric mixture after chromatographic purification). By reaction with the acyl-nitroso derivative BnOCON=O prepared in situ by oxidation of benzyl *N*-hydroxycarbamate with an ammonium periodate, the diene **5b** gave the Diels–Alder adduct **6** as a non-re-

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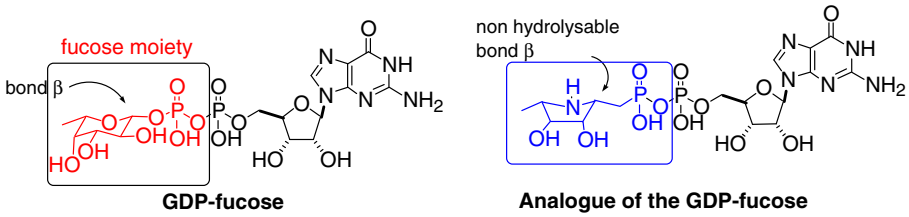
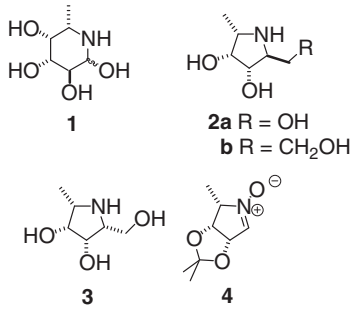
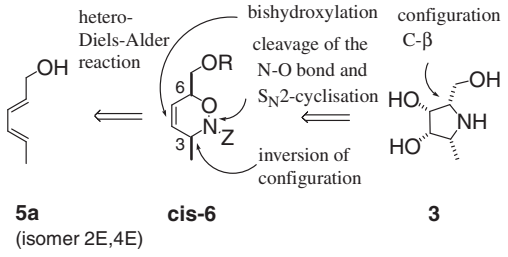



**Scheme 1.**




**Scheme 2.**

solved isomeric mixture with mainly *cis*-configuration. Without purification, the adduct mixture **6** was *cis*-bishydroxylated under standard conditions with osmic oxide/*N*-methyl morpholine oxide (NMO) to give a mixture of bishydroxylated isomers. The major diol **7** (allitol series) was easily isolated by crystallisation in diethyl ether as pure compound in a 32% overall yield from the sorbic alcohol **5a**. The stereo-structure of diol **7** was ascertained with NMR spectroscopy by comparison of the interproton couplings with those of similar compounds, described previously.[16]



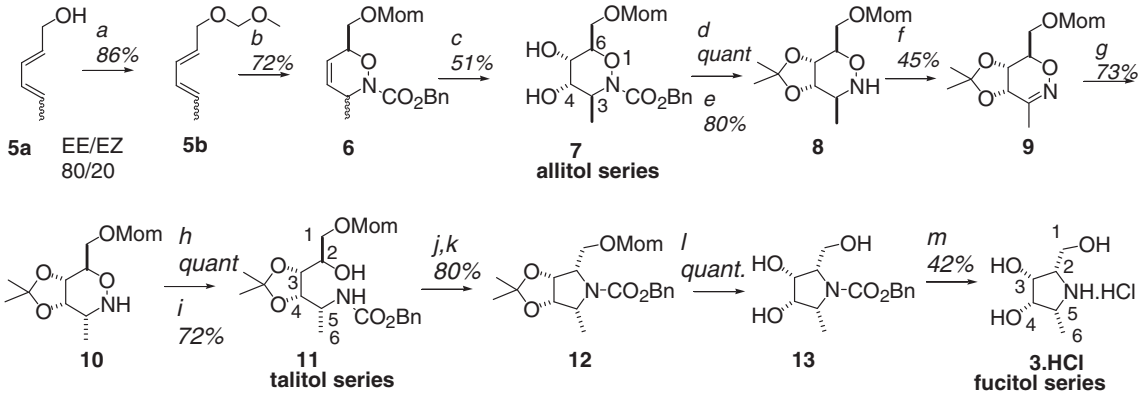

**Scheme 3.**

The next important step was the inversion of the configuration at C-3 as following. The diol moiety was protected as acetonide in dimethoxypropane catalysed with acidic resin. After N-deprotection by hydrogenolysis over palladium, the cyclic hydroxylamine function was oxidised by successive reaction with *N*-chlorosuccinimide and elimination with DBU in cyclohexane, according to a previously used method,[17] to give the cyclic oxime **9** in a 36% overall yield from the diol **7**. The reduction with sodium cyanoborohydride in dichloromethane containing acetic acid provided the inverted cyclic hydroxylamine **10**.

The oxazinane ring opening was easily carried out by hydrogenolysis over Raney-nickel at room temperature and N-protection with benzyl chloroformate to give the protected linear 5-aminotalitol **11**. This was cyclised into pyrrolidine by a previously described method:[22] The free hydroxyl group was mesylated and the cyclisation by $S_N2$ reaction with the amide function occurred in ethanolic NaOH at 80 °C to give the protected amino-fucitol **12**. Acidic O-deprotection yielded the N-protected amino-fucitol **13**, and subsequent N-deprotection by hydrogenolysis over palladium in the presence of hydrochloric acid led to the final 2,5-imino-fucitol **3**·HCl as crystalline hydrochloride in a 25% overall yield from the cyclic oxime **9**.

NMR spectra of the pyrrolidine **3** in $D_2O$ are pH-sensitive and were measured at natural pH (ca. 5)[23] for the hydrochloride salt and at pH 8–10 for the free base. $^1H$ and $^{13}C$ NMR data were in agreement with those of Clapès[20] but not with those of Asano[19] whose material was probably another isomer.

The inhibition data of imino-fucitol **3** against representative members of the glycosidase family[24] are reported in Table 1, accompanied by those for the C-α-anomers D-**2a**, D-**14**[22] and L-**2a**,[21] L-**2b**[14] (Scheme 5). The data for the basic homologues D-**15**[25] and L-**16**,[17] respectively, D-galactose and L-fucose analogues are given as well. The *all-cis* racemic compound **3** is a very potent

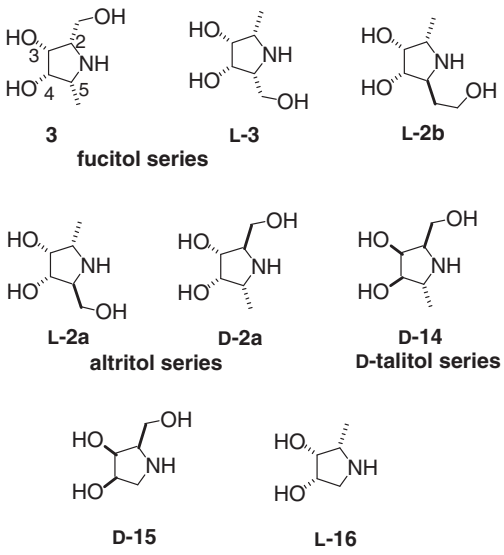



**Scheme 4.** Reagents and conditions: (a) $ClCH_2OMe$, $NEtiPr_2$; (b) $BnMe_3NIO_4$, BnOCONHOH, rt, 1 h; (c) $OsO_4$ cat., NMO, $H_2O$–acetone, 40 °C, 16 h; (d) dimethoxypropane, Amberlyst 15 ($H^+$), 40 °C, 2 h; (e) $H_2$/Pd–C, 1 h; (f) NCS 1.5 equiv, then DBU, 1.8 equiv, cyclohexane; (g) $NaBH_3CN$, $AcOH/CH_2Cl_2$ 1:3, 1.5 h; (h) $H_2$–Raney-nickel, EtOH, 16 h; (i) $ClCO_2Bn$, NaOH N, 1 h; (j) $MeSO_2Cl$, $NEt_3$, $CH_2Cl_2$, 4 h; (k) NaOH 2.5 N, EtOH, 16 h, 80 °C; (l) HCl 2 N, EtOH, 24 h, 30 °C; (m) $H_2$/Pd–C, EtOH, 1 equiv HCl.

**Table 1**
Inhibition constants ($K_i$) for pyrrolidines racemic **3** and L-**3**,[21] L-**2a**,[21] D-**2a**,[22] L-**2b**,[14] D-**14**,[22] D-**15**[25] and L-**16**[17] against representative members of glycosidase family[a]

| Enzymes | α-Gluc'ase | β-Gluc'ase | α-Man'ase | β-Man'ase | α-Galac'ase | β-Galac'ase | α-Fuc'ase |
|---|---|---|---|---|---|---|---|
| (±)-**3**[b] | >100 μM[c] | >1 mM[c] | >1 mM[c] | >1 mM[c] | 3 μM | 53 μM | 15 nM |
| L-**3**[21] | | | | | | | 4.9 nM |
| D-**2a**[22] | ni | 100 μM[c] | ni | nd | 2 mM[c] | nd | 1 mM |
| L-**2a**[21] | | | | | | | 80 nM |
| L-**2b**[14] | >100 μM[b,c,d] | >1 mM[b,c] | >1 mM[b,c] | >1 mM[b,c] | >1 mM[b,c] | >1 mM[b,c] | 8 nM |
| D-**14**[22] | ni | 1.2 mM[c] | 53 μM | nd | 5 μM | nd | 9 μM |
| D-**15**[25] | ni | 350 μM[c] | 14 μM[c] | | 0.2 μM[c] | 140 μM[c] | ni |
| L-**16**[17] | | | | | | | 50 nM |

[a] ni, non inhibition; nd, non determined.
[b] Present work.
[c] $IC_{50}$.
[d] Slow binding.




**Scheme 5.**

fucosidase inhibitor ($K_i$ 15 nM) with a large specificity for this particular enzyme, α- and β-galactosidases being only moderately inhibited ($K_i$ = 3–50 μM). The enantiomer L-**3**, having the configuration of the L-fucose at C-3, C-4, C-5 and of the D-galactose at C-2, C-3, C-4, seems to be responsible for this high affinity by comparison with the potent fucosidase inhibitors L-**2a**[21] and L-**2b**[14] and with the moderate galactosidase inhibitor D-**14**.[22] Inversion of the fucose configuration in the D-series for D-**2a**[22] led to a lack of inhibition of the fucosidase. The inhibition value of the enantiomer L-**3**, mentioned by Clapès,[21] is in agreement with our present results.

The comparison of the inhibition values of these 2,5-substituted pyrrolidinetriols with those of the simpler aminolyxitols D-**15** and L-**16** permits to state more precisely the importance of the 5-methyl and the 2-$CH_2OH$ groups in interaction with the studied glycosidases. The configuration of the 5-methyl group in *syn* relationship with the *cis*-diol moiety appears to be of prime importance for the recognition of **3**, L-**3**, L-**2a**, L-**2b** by the fucosidase tested. By contrast, in comparison with the 5-deoxyaminolyxitol L-**16**, the presence of the 2-$CH_2OH$ group (or the 2-$CH_2CH_2OH$ group in L-**2b**) is a favourable element, but its configuration does not seem so important for binding. Concerning the galactosidases, the configuration of the 5-methyl group clearly proved not to be essential, contrary to the *syn* relationship between the diol moiety and the $CH_2OH$ group which is necessary. Compared with the D-lyxitol D-**15**, the methyl group in **3** appeared to have a weak influence, favourable in the case of the α-galactosidase and unfavourable in the case of the β-galactosidase. The importance of the configuration of these groups concerning the glycosidase inhibitions has already been y discussed.[26]

We have set up the first synthesis of the *all-cis* iminofucitol **3** as racemic compound in 3% overall yield from the inexpensive sorbic alcohol **5a**. This compound turned out to be a powerful L-fucosidase inhibitor and a weaker but still significant D-galactosidases inhibitor. Our synthetic method offers an effective route to fucose analogues of the C-β-anomeric series. It has, therefore, a strong potential for further chemical elaboration and derivatisation.

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## Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.10.043.

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