



Inhibition of Fucosyl Transferase and Fucosidase by a Rigid Bicyclic Mimic of α -L-Fucose

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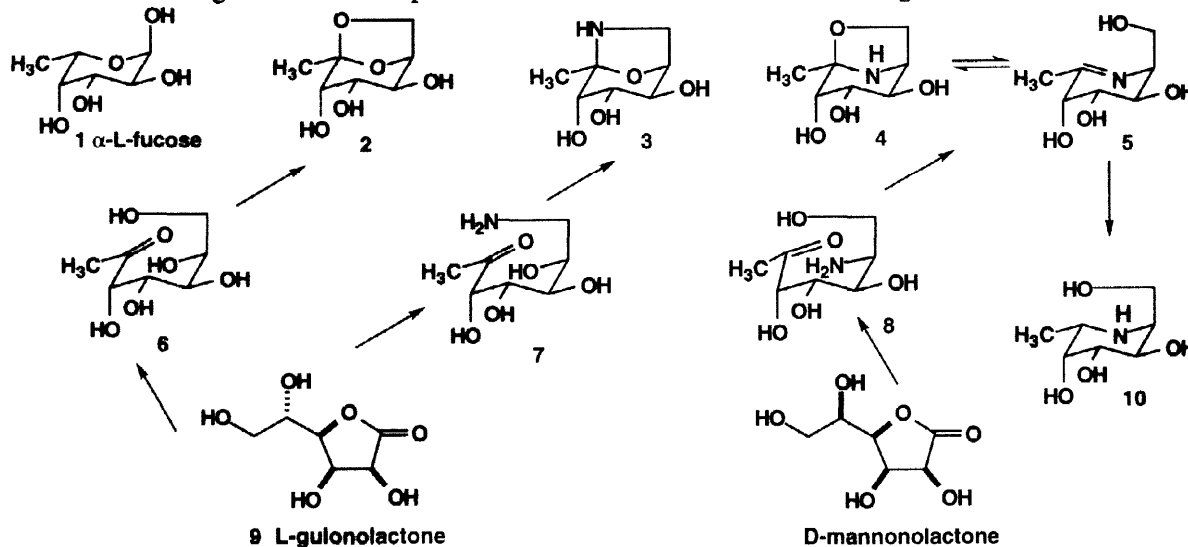
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Abstract: A rigid bicyclic analogue of α -L-fucose, prepared from a monoacetonide of L-gulonolactone in an overall yield of 36% without the need for column chromatography, is a potent inhibitor of fucosidases and a weak inhibitor of a fucosyl transferase.

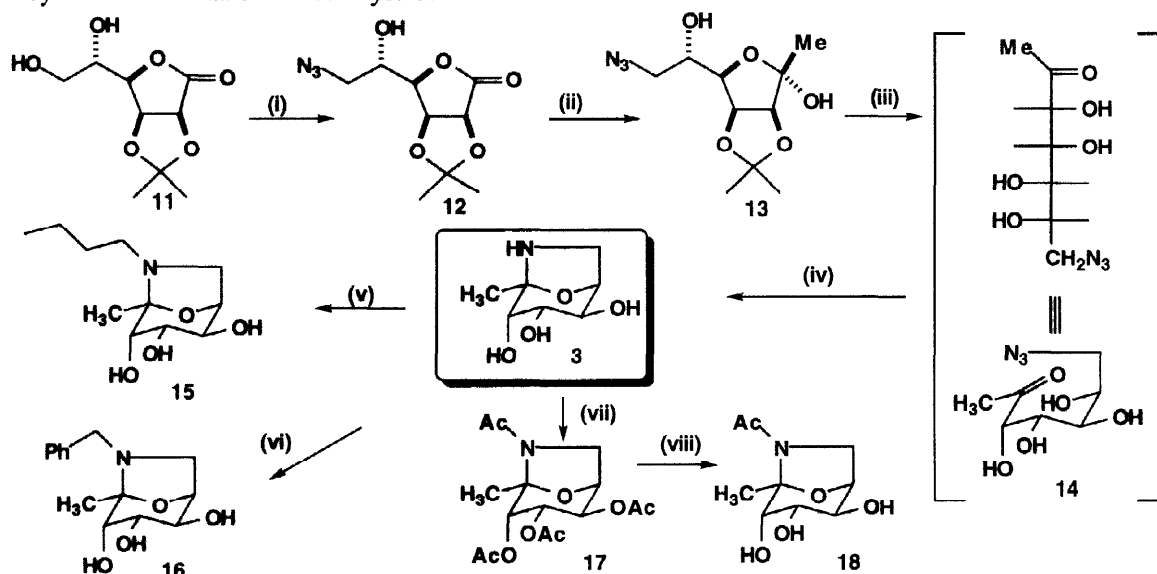
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Analogues of L-fucose **1** may inhibit enzymes which hydrolyse (fucosidase)¹ or make (fucosyl transferases)² oligosaccharides, or may bind to receptors which are important components of cell-cell recognition such as Lewis x.³ There are an enormous number of potential applications of materials that have specific interactions in any of these biological processes, not only as potential chemotherapeutic agents but more certainly as probes for the understanding of a number of processes in which the immunodominant sugar L-fucose is involved.⁴



The bicyclic anhydrosugar **2**⁵ is a conformationally fixed mimic of α -L-fucose **1**, in which the three hydroxyl groups of the fucopyranose ring [which appear to be a pre-requisite for fucose recognition in enzymes and receptors⁶] are present. The ease of synthesis and the stability of the intramolecular ketal **2** [formed by dehydration of the heptulose **6**, itself efficiently prepared from L-gulonolactone **9**] indicated that the hemiaminals **3** and **4** might also be stable. Such structures should constitute a new set of fucose mimics with interesting biological properties; interactions between a protonated nitrogen and the enzyme or receptor might provide strong binding of the mimic. The hemiaminals **3** and **4** should be accessible by dehydration of aminoketones **7** and **8**, derivable from the readily available epimeric L-gulo- and D-manno-lactones, respectively. This paper reports the synthesis of the stable **3** from **9** in an overall yield of 36% in a sequence that avoids any chromatography; **3** is a potent inhibitor of fucosidases and a weak inhibitor of a fucosyl transferase. Both N-alkyl and N-acyl derivatives of **3** are easily prepared. In contrast, the isomeric bicyclic amine **4** is in equilibrium with significant amount of the monocyclic imine **5** and is relatively unstable; the mixture of **4** and **5** causes no inhibition of fucosidases, surprising in that further reduction of the mixture gives α -homofuconojirimycin **10**, the most potent inhibitor of fucosidases yet reported.⁷

For the synthesis of **3**, L-gulonolactone **9** was first converted into the easily crystallised monoacetone **11**, m.p. 153-154°C, $[\alpha]_{\text{D}}^{20} +75.8$ (*c.* 1.1 in CHCl_3) [lit.⁸ 153-154°C, $[\alpha]_{\text{D}}^{24} +91.5$ (*c.* 1.0 in CHCl_3)] [Scheme 1]. Selective esterification of the primary hydroxyl group in **11** by methanesulfonyl chloride in pyridine and dichloromethane, followed by treatment of the resulting mesylate with sodium azide in DMF, gave the azide **12**, oil, $[\alpha]_{\text{D}}^{22} +33.9$ (*c.* 1.0 in CHCl_3), in an overall yield of 61%. Addition of methyl lithium to the azidolactone **12** in tetrahydrofuran afforded, after work-up, a single crystalline lactol **13**,⁹ m.p. 91-93°C, $[\alpha]_{\text{D}}^{21} +4.4$ (*c.* 1.0 in CHCl_3), 68% yield. Removal of the isopropylidene group in **13** by hydrolysis with Amberlite IR-120 (H+) ion exchange resin gave the azido heptulose **14** [drawn as an open chain structure but which exists as a mixture of anomeric cyclic forms]. Hydrogenation of the hydrolysate in the presence of palladium on charcoal followed by purification by ion exchange chromatography gave the bicyclic hemiaminal **3**¹⁰ in 85% yield.

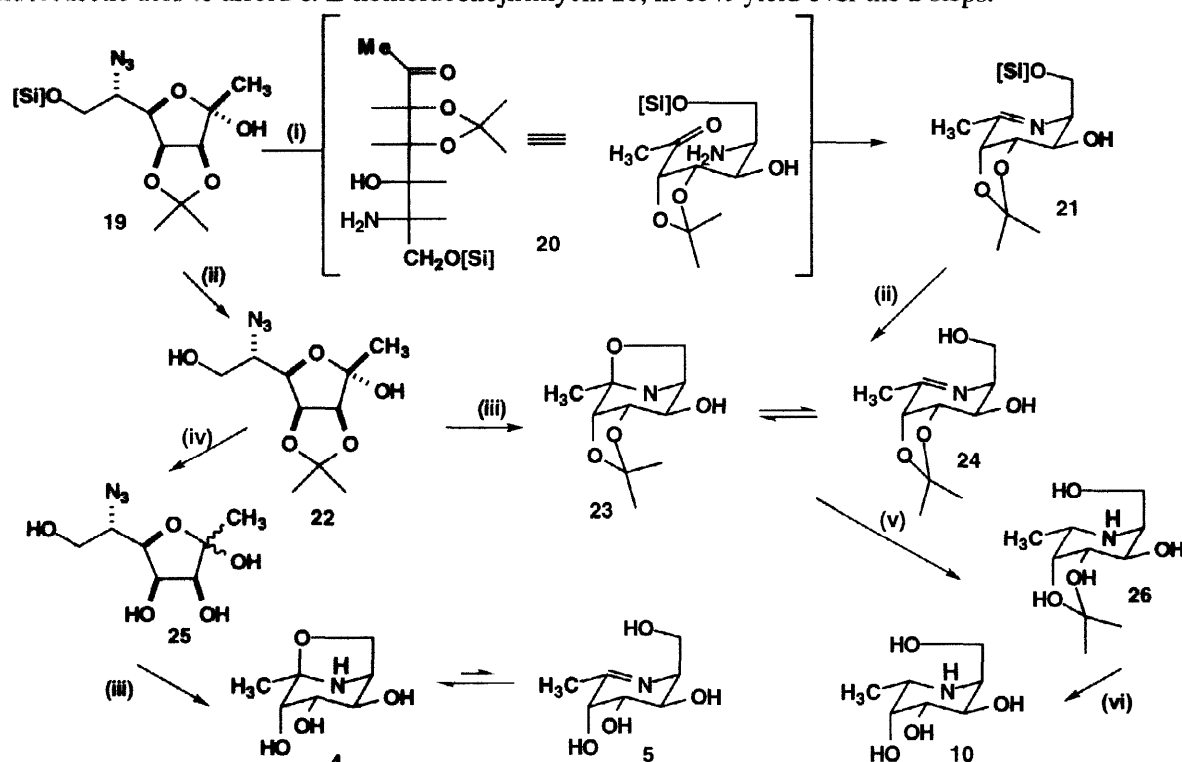


Scheme 1: (i) MeSO_2Cl , CH_2Cl_2 , pyridine, -20°C ; then NaN_3 , DMF (ii) MeLi , THF, -70°C (iii) Amberlite IR-120, H^+ form, H_2O (iv) H_2 , Pd/C , H_2O (v) *n*-PrCHO, H_2 , Pd black, MeOH (vi) PhCH_2Br , K_2CO_3 , DMF (vii) Ac_2O , pyridine (viii) Et_3N , MeOH

Derivatives of **3** bearing an alkyl or acyl group on the nitrogen were easily prepared. Thus, hydrogenation of **3** with butyraldehyde in the presence of palladium on carbon in methanol resulted in reductive amination to afford the *N*-butyl analogue **15**, m.p. 139-140°C, $[\alpha]_{\text{D}}^{21} -84.2$ (*c.* 0.53 in MeOH) while alkylation of **3** with benzyl bromide in dimethylformamide gave the *N*-benzyl amine **16**, m.p. 190-191°C, $[\alpha]_{\text{D}}^{22} -54.5$ (*c.* 0.62 in MeOH); Peracetylation of **3** with acetic anhydride in pyridine gave the tetraacetate **17**, m.p. 134-135°C, $[\alpha]_{\text{D}}^{24} -63.2$ (*c.* 1.1 in CHCl_3), which with basic methanol gave the *N*-acetylated triol **18**, m.p. 178-180°C, $[\alpha]_{\text{D}}^{24} -73.5$ (*c.* 1.15 in EtOH). Thus, the bicycle with the nitrogen in the bridge of the fucose system **3** gave stable derivatives suitable for biological evaluation in short efficient synthetic sequences.

The azidolactol **19**, prepared from D-mannonolactone as previously described,¹¹ was a key intermediate in the synthesis of the isomeric amine **4** which contains a piperidine moiety [Scheme 2]. Initial hydrogenation of **19** in ethyl acetate in the presence of palladium black gave the corresponding amine **20** which spontaneously cyclised to form the stable imine **21**, m.p. 94-96°C, $[\alpha]_{\text{D}}^{20} -48.4$ (*c.* 1.0 in CHCl_3), in 69% yield. Desilylation of **21** with tetrabutyl ammonium fluoride in THF gave an equilibrium mixture¹² of the intramolecular hemiaminal **23** and the imine **24** in 99% yield. The composition of this mixture was solvent dependent, with the bicyclic form **23** predominating in CD_3CN and D_2O , whilst in d_6 -DMSO both components were present in roughly equal amounts. The same equilibrium mixture of **23** and **24** was also

formed, in 90% yield, by hydrogenation of the desilylated azidolactol **22**. in ethanol with a catalyst of palladium on carbon. Further hydrogenation of **23** and **24** in ethanol in the presence of Adams' catalyst gave the acetoneide **26** from which the isopropylidene group was removed by acid hydrolysis with aqueous trifluoroacetic acid to afford α -L-homofuconojirimycin **10**, in 85% yield over the 2 steps.



Scheme 2: (i) H_2 , Pd black, EtOAc (ii) $Bu_4N^+F^-$, THF (iii) H_2 , Pd/C, EtOH (iv) Amberlite IR-120, H^+ form, H_2O (v) H_2 , PtO_2 , EtOH (vi) CF_3COOH , H_2O

All attempts to remove the isopropylidene group from the mixture of **23** and **24** by acid hydrolysis were unsuccessful, indicating that the bicyclic amine **4** was sensitive to acid hydrolysis. Accordingly, the isopropylidene group was removed from **22** in 88% yield by hydrolysis with acidic ion exchange resin. Partial hydrogenation in ethanol in the presence of palladium on carbon of the mixture of unprotected lactols **25**, $[\alpha]_D^{25} +2.2 \rightarrow +41.5$ (24 h) (c , 1.0 in EtOH), gave the unstable bicyclic system **4**, which existed in equilibrium with a small amount of the open imine **5**.¹³ Unlike a number of 5 ring sugar imines,¹⁴ this system showed no tendency to hydrate or to dimerise in solution. However, over a period of days, **4** decomposed into a complex mixture of products.

Preliminary investigations of the effects of both bicyclic systems **3** and **4** on human liver glycosidases, other glycosidases and a fucosyl transferase were conducted. The stable bicyclic amine **3** was a powerful and selective inhibitor of fucosidases, with a K_i value of $6 \mu M$ against human liver α -fucosidase and a K_i value of $68 \mu M$ against human placenta α -fucosidase; there was no significant inhibition of any other glycosidase.¹⁵ Thus the bicyclic mimic **3** is as potent as the best pyrrolidine fucosidase inhibitors,¹⁶ although it is much less potent than either α -L-homofuconojirimycin **10** or deoxyfuconojirimycin.¹⁷ There was also some inhibition of human α -3 fucosyl transferase¹⁸ by **3** with an IC_{50} of approximately 35 mM based on 60% inhibition at 50 mM and 36% inhibition at 25 mM¹⁹. The *N*-acetylated amine **18** had no effect on any of the enzymes studied indicating that a basic site may be helpful in increasing binding between the bicyclic fucose mimics and the target enzymes. Although *N*-alkylation has significant effect on many biological properties of amino sugars,²⁰ no enhancement of either the fucosidase or transferase inhibition was found with the *N*-butyl **15** and *N*-benzyl **16** derivatives.

In contrast, the relatively unstable bicyclic system **4** showed no inhibition of any of the glycosidases. Since the further reduction product of **4** is the very powerful inhibitor **10** and the open chain form **5** contains a piperidine ring with admittedly a rather non-basic imine nitrogen, this is somewhat surprising. It also shows that there is no trace of the fully reduced piperidine **10** in the samples prepared of the mixture of **4** and **5**. In any event, the chemical instability of **4** makes this an unattractive class of compounds to investigate further as potential biological probes. In summary, this paper shows that rigid bicyclic analogues of L-fucose are readily available, and constitute a new class of carbohydrate mimics with novel biological properties.²¹

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9. Assignment of the anomeric configuration of lactol **13** is based on nOe experiments which will be reported in a full paper.
10. Selected data for **3**: foam, $[\alpha]_{\text{D}}^{21}$ -59.0 (c, 1.1 in H₂O) δ_{H} (D₂O, 500 MHz, pH 9): 1.51 (3H, s, CH₃), 2.95 (1H, d, H-7, *J*_{7,7'} 10.8 Hz), 3.10 (1H, dd, H-7', *J*_{7,7'} 7 10.7 Hz, *J*_{7,6} 6.5 Hz), 3.60 (1H, d, H-3, *J*_{3,4} 4.3 Hz), 3.65 (1H, dd, H-4, *J*_{4,5} 9.3 Hz, *J*_{4,3} 4.4 Hz), 3.77 (1H, dd, H-5, *J*_{5,4} 9.4 Hz, *J*_{5,6} 3.9 Hz), 4.38 (1H, dd, H-6, *J*_{6,7'} 6.2 Hz, *J*_{6,5} 4.0 Hz); δ_{C} (D₂O, 125 MHz, pH 9): 20.7 (q, C-1), 44.8 (t, C-7), 70.7, 70.8, 75.1, 76.5 (4 x d, C-3, C-4, C-5, C-6), 96.0 (s, C-2).
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12. Selected data for bicyclic amine **23** and imine **24**: δ_{H} (d₆-DMSO, 500 MHz + COSY): **23**: 1.23, 1.34, 1.37 (3 x 3H, 3 x s, 3 x CH₃), 3.15 (1H, br s, NH, D₂O exchanges), 3.28 (1H, dd, H-7, *J*_{7,7'} 7.0 Hz, *J*_{7,6} 5.6 Hz), 3.38 (1H, t, H-6, *J* 4.7 Hz), 3.57-3.58 (1H, m, H-5), 3.66 (1H, d, H-7', *J*_{7,7'} 7.3 Hz), 3.81-3.84 (2H, m, H-3, H-4), 5.13 (1H, d, OH-5, *J* 4.2 Hz); **24**: 1.27, 1.30 (2 x 3H, 2 x s, (CH₃)₂C), 1.95 (3H, d, C-1, ³*J* 1.9 Hz), 3.32-3.33 (1H, m, H-6), 3.54 (1H, dd, H-7, *J*_{7,7'} 10.6 Hz, *J*_{7,6} 7.5 Hz), 3.70 (1H, dd, H-7', *J*_{7,7'} 10.5 Hz, *J*_{7,6} 4.9 Hz), 3.75 (1H, dd, H-5, *J*_{5,4} 4.7 Hz, *J*_{5,6} 3.3 Hz), 4.23 (1H, dd, H-4, *J*_{4,3} 6.5 Hz, *J*_{4,5} 4.8 Hz), 4.29 (1H, d, H-3, *J*_{3,4} 6.5 Hz), 4.54 (1H, t, OH-7, *J* 5.1 Hz, D₂O exchanges), 5.01 (1H, d, OH-5, *J* 4.6 Hz, D₂O exchanges); δ_{C} (CD₃CN, 125 MHz): **23** (major component): 21.6, 26.5, 28.3 (3 x q, C-1, (CH₃)₂C), 61.0 (d, C-6), 65.1 (t, C-7), 74.3, 80.2, 80.8 (3 x d, C-3, C-4, C-5), 94.4 (s, C-2), 111.2 (s, (CH₃)₂C); **24** (minor component): 24.3, 25.6, 27.2 (3 x q, C-1, (CH₃)₂C), 59.3 (d, C-6), 63.7 (t, C-7), 68.3, 72.6, 75.8 (3 x d, C-3, C-4, C-5), 110.2 (s, (CH₃)₂C), 168.0 (s, C=N).
13. Selected data for **4** and **5**: δ_{H} (D₂O): 1.26 (2.8H, s, CH₃), 1.85 (0.2H, d, *J* 1.7 Hz), 3.30-3.49 (4H, m), 3.52-3.57 (1H, m), 3.70 (1H, d, *J* 8.0 Hz); **4** (major component); m.p. 99-102 °C (dec.), $[\alpha]_{\text{D}}^{25}$ -8.9 (c, 0.94 in H₂O) δ_{C} (D₂O, 125 MHz, pH 9): 21.0 (q C-1), 66.2 (t, C-7), 59.8, 71.8, 72.7, 74.4 (4 x d, C-3, C-4, C-5, C-6), 97.6 (s, C-2); **5** (minor component); 19.8 (q, C-1), 67.6 (t, C-7), 60.1, 73.7, 77.0 (3 x d, C-3, C-4, C-5, C-6).
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15. No significant inhibition at 1.0 mM **3** was found for yeast α -glucosidase, almond β -glucosidase, green coffee bean and *E. coli* α -galactosidases, Jack bean α -mannosidase, bovine kidney and Jack bean α -N-acetylglucosaminidases, *Penicillium decumbens* α -L-naringinase. For details of the assays, see A.A. Watson, R.J. Nash, M.R. Wormald, D.J. Harvey, S. Dealler, E. Lees, N. Asano, H. Kizu, R.C. Griffiths, A.J. Cairns, G.W.J. Fleet, *Phytochemistry*, **1997**, *46*, 255.
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21. All new compounds in this paper have spectral and other physical data consistent with the structures proposed and satisfactory CHN microanalyses have been obtained for **12 13 15 16 17 18 19 21 22 23** and **25**. This work was supported by CASE and fully funded studentships with GlaxoWellcome, and by an EPSRC post doctoral fellowship.