Tetrahedron: *Asymmetry* 20 (2009) 744–745

Contents lists available at ScienceDirect

Tetrahedron: *Asymmetry*

journal homepage: www.elsevier.com/locate/tetasy

Solid-phase supported mimic of GDP-L-galactose

Barbara La Ferla, Laura Russo, Cristina Airoldi, Francesco Nicotra *

Department of Biotechnology and Bioscience, University of Milano Bicocca, Piazza della Scienza 2, I-20126 Milano, Italy

ARTICLE INFO

Article history:
Received 26 February 2009
Accepted 4 March 2009
Available online 30 March 2009

This paper is dedicated to Professor George Fleet, on the occasion of his 65th birthday

ABSTRACT

A C-glycoside mimetic of L-galactose 1-diphosphate, a potential ligand of GDP-L-galactose hydrolase, an enzyme involved in the biosynthesis of vitamin C, has been designed, stereoselectively synthesised by C-allylation of tribenzylated L-fucose, by periodate-osmium tetroxide degradation of the double bond, by condensation of the obtained aldehydes with benzylacetate and by deprotection. The obtained mimetic was linked to a sepharose resin.

1. Introduction

The world production of vitamin C (L-ascorbic acid) is around 80000 tons/year, and is obtained from D-glucose exploiting a synthetic procedure developed by Reichstein in 1933 with few subsequent improvements.[1] In light of the economical relevance of this compound considerable efforts are being made to develop biotechnological procedures, which would allow vitamin C to be produced by fermentation, and therefore the enzymes involved in its biosynthesis are relevant targets.

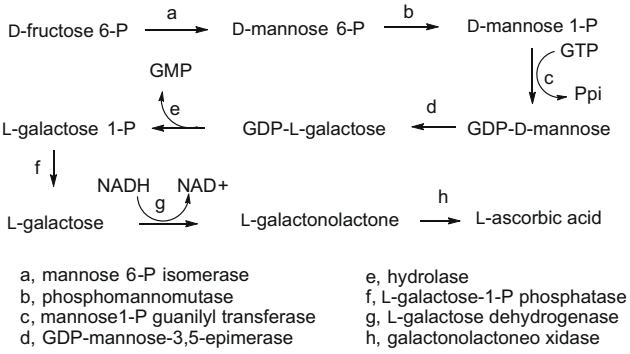
The biosynthesis of L-ascorbic acid in vegetal organisms has been extensively studied. The process starts from D-glucose, which is converted into D-fructose 6-phosphate, which in turn undergoes the transformations reported in Scheme 1.

The enzymes involved in this pathway have been isolated and cloned, but one of them, the phosphohydrolase, which converts GDP-L-galactose into L-galactose 1-phosphate, was unknown until very recently, and is now the subject of great interest.[2]

In this context, we planned the synthesis of stable mimics of GDP-galactose or L-galactose 1-phosphate with the aim of not only facilitating the purification of the enzyme by affinity chromatography, but also to find an artificial ligand that could eventually act as an inhibitor or as a chaperone.[3]

L-Galactose is a rare sugar, which in not commercially available, therefore we decided to take advantage on the structural similarity with L-fucose, and designed the L-fuco derivative **1** as mimic of the L-galactose 1-diphosphate moiety of GDP-L-Gal (Fig. 1).

The C-glycosidic nature of compound **1** guarantees its stability, the two oxygen atoms, properly positioned to chelate Mg^{2+} favour the interaction with the active site of the enzyme, and the carboxylic function makes the compound suitable for ligation to a resin.



Scheme 1.

2. Results and discussion

The synthesis of compound **1** was performed starting from commercially available 2,3,4-tri-O-benzyl L-fucose, which, after acetylation, was submitted to a Sakurai reaction with allyltrimethylsilane ($BF_3{\cdot}OEt_2$, MeCN, 98% yield) to stereoselectively afford 2-(tri-*O*-benzyl-α-L-fucopyranosyl)-1-propene **2**[4] (no traces

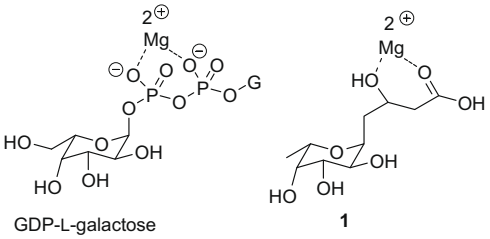
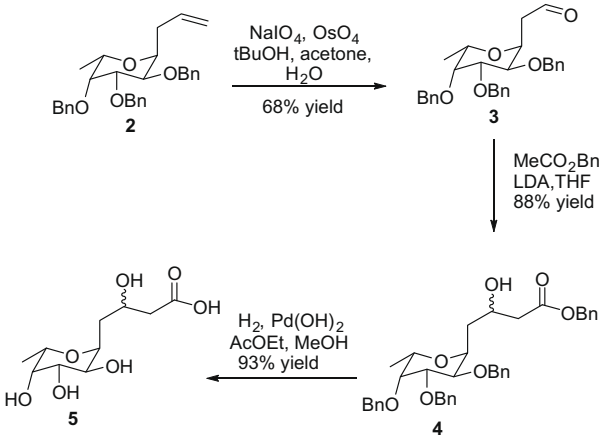


Figure 1.

* Corresponding author.
E-mail address: francesco.nicotra@unimib.it (F. Nicotra).

0957-4166/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetasy.2009.03.005

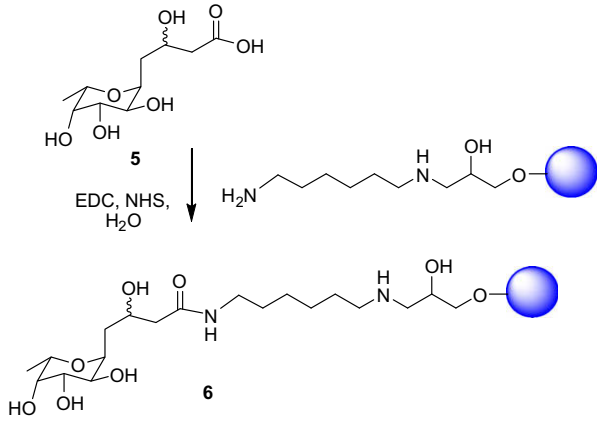
of the β anomer were detected). This procedure improves that described in the literature,[5] in which compound **2** was obtained by the acetylation of L-fucose, a Sakurai reaction, saponification of the acetates and finally benzylation (Scheme 2).



Scheme 2.

2-(Tri-*O*-benzyl-α-L-fucopyranosyl)-1-propene **2** was submitted to periodate-osmium tetroxide cleavage of the double bond and the aldehyde obtained, 2-(tri-*O*-benzyl-α-L-fucopyranosyl)-1-acetaldehyde **3**[6] (68% yield) was condensed with benzyl acetate (LDA, THF, 88% yield) in order to generate benzyl 4-(tri-*O*-benzyl-α-L-fucopyranosyl)-3-hydroxybutanoate **4**[7] as a mixture of epimers (1.1/0.9 ratio). This mixture was not separated since both stereoisomers were expected to be ligands of the target enzyme. Catalytic hydrogenation (H_2, $Pd(OH)_2$, AcOH–MeOH, 96% yield) afforded the completely deprotected 4-(α-L-fucopyranosyl)-3-hydroxybutanoic acid **5**,[8] the L-galactose 1-diphosphate mimetic and potential ligand of GDP-galactose phosphohydrolase.

Compound **5** was finally supported on an EAH Sepharose resin (–NH_2 functionalised, see Scheme 3) by activation of the carboxylic acid with 1-ethyl-3-dimethylaminopropyl-carbodiimide (EDC) and *N*-hydroxysuccinimide, performing the reaction in water.



Scheme 3.

The functionalised resin **6** obtained was submitted to the colorimetric TNBS test[9] which showed the absence of residual free amino groups, therefore the loading was 0.11 mol of compound **5** per ml of resin. IR analysis confirmed the presence of the ligand (Fig. 2).

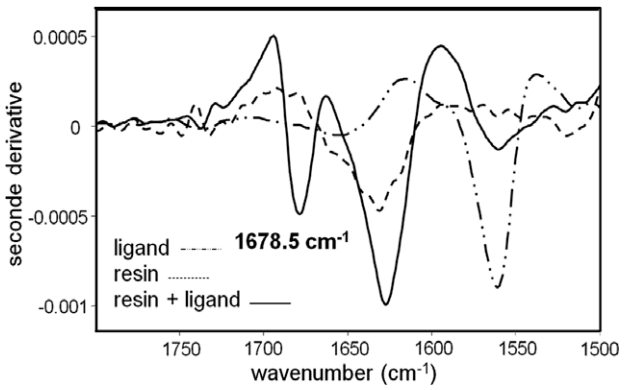


Figure 2. Second derivative of IR absorption spectra of ligand, resin before coupling reaction and resin after coupling reaction. Spectra were collected in ATR-FTIR (Attenuated Total Reflectance-Fourier Transform Infrared Spectroscopy) at a resolution of 2 cm^{-1}.

3. Conclusion

A mimic of L-galactose 1-diphosphate, a potential ligand of the enzyme that converts GDP-L-galactose into L-galactose 1-phosphate was designed, synthesised stereoselectively and supported on a sepharose resin as an interesting tool to isolate and purify the enzyme, and to study the structural requirements for good inhibitors/chaperones.

References

1. Reichstein, T. U.S. Patent 2,265,121, 1941 and 2,301,811, 1942.
2. Linster, C. L.; Adler, L. N.; Webb, K.; Christensen, K. C.; Brenner, C.; Clarke, S. G. *J. Biol. Chem.* **2008**, *283*, 18483.
3. For a review on the role of glycomimetics as chaperons for refolding of carbohydrate processing enzyme see: Fan, J.-Q. Iminosugars as Active-Site-Specific Chaperones for the Treatment of Lysosomal Storage Disorders in Iminosugars. Compain, P., Martin, O. R., Eds.; John Wiley & Sons Ltd: Chichester, UK, 2007, pp 225–247.
4. *Selected analytical data of 3-(tri-O-benzyl-α-L-fucopyranosyl)-1-propene* **2**: ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.41–7.21 (m, 5H, HAr), 5.87–5.71 (m, 1H, H(2′)), 5.16–4.98 (m, 2H, H83′a),(3′b)), 4.81–4.52 (m, 6H, 6C*H*Ph), 4.16–4.04 (m, 1H, H(1)), 4.04–3.94 (m, 1H, H(5)), 3.85–3.74 (m, 3H, H(2),(3),(4)), 2.50–2.27 (m, 2H, H(1′a),(1′b)), 1.31 (d, J = 6.6 Hz, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 139.0, 138.8, 138.6, 135.6, 129.0–127.8, 116.9, 77.12, 76.82, 76.03, 73.32, 73.23, 73.23, 70.41, 68.93, 15.43; $[\alpha]_D^{20} = -16.6$ (c 1.5, $CHCl_3$).
5. Uchiyama, T.; Woltering, T. J.; Wong, W.; Lin, C.-C.; Kaijmoto, T.; Takebayashi, M.; Weitz-Schmidt, G.; Asakura, T.; Noda, M.; Wong, C.-H. *Bioorg. Med. Chem.* **1996**, *4*, 1149.
6. *Selected analytical data of 2-(tri-O-benzyl-α-L-fucopyranosyl)-1-acetaldehyde* **3**: ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 9.69 (m, 1H, C*H*O), 7.41–7.21 (m, 5H, HAr), 4.77 (d, J = 11.92 Hz, 2H, 2C*H*Ph), 4.72–4.59 (m, 4H, 3C*H*Ph, H(1)), 4.50 (d, J = 11.71 Hz, 1H, C*H*Ph), 3.96–3.89 (m, 1H, H(5)), 3.86 (dd, J = 6.5, 4.2 Hz, 1H, H(2)), 3.79–3.77 (m, 1H, H(4)), 3.75 (dd, J = 6.5, 2.9 Hz, 1H, H(3)), 2.68–2.58 (m, 2H, H(1′a), H(1′b), 1.29 (d, J = 6.7 Hz, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 201.2, 138.7, 138.6, 138.1, 129.0–127.8, 77.24, 76.35, 75.74, 73.52, 73.462, 73.280, 69.54, 67.04, 43.10, 15.55; $[\alpha]_D^{20} = -26.1$ (c 1.8, $CHCl_3$).
7. *Selected analytical data for benzyl 4-(tri-O-benzyl-α-L-fucopyranosyl)-3-hydroxybutanoate* **4**: (* isomer signals) ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.53–7.11 (m, HAr), 5.16 (br s, CH_2Ph), 4.82–4.45 (m, 6C*H*Ph), 4.37–4.29 (m, H(2′)), 4.29–4.15 (m, H(2′*),(1),(1*)), 4.09–3.99 (m, H(5*), 3.97–3.85 (m, H(5)), 3.83–3.70 (m, H(2),(3),(4),(2*),(3*),(4*)), 3.65–3.59 (m, OH), 3.14–3.06 (m, OH*), 2.63–2.43 (m, H(1′a),(1′b),(1′a*),(1′b*)), 1.97–1.80 (m, H(3′a),(3′a*)), 1.69–1.54 (m, H(3′b),(3′b*)), 1.36–1.26 (m, CH_3, CH_3^*); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.6, 172.0, 138.9, 138.8, 138.7, 138.6, 138.3, 138.3, 136.0, 135.9, 129.0–127.8, 77.15, 77.11, 76.88, 76.84, 75.97, 75.94, 75.69, 75.67, 75.64, 75.61, 73.48, 73.40, 73.36, 73.31, 73.28, 73.22, 69.45, 69.15, 68.42, 66.67, 66.60, 65.70, 42.03, 41.95, 15.51, 15.47.
8. *Selected analytical data for 4-(L-fucopyranosyl)-3-hydroxybutanoic acid* **5**: ^{1}H NMR (400 MHz, CD_3OD) δ ppm 4.23–4.05 (m, H(1), (1*),(2′),(2′*), 3.95–3.77 (m, H(2),(5),(2*),(5*), 3.74–3.54 (m, H(3),(4),(3*),(4*)), 2.57 (dd, J = 15.4, 4.3 Hz, H(1′a)), 2.52–2.45 (m, H(1′a*),(1′b*)), 2.39 (dd, J = 15.4, 8.6 Hz, H(1′b)), 2.00–1.76 (m, H(3′a),(3′b),(3′a*)), 1.73–1.63 (m, H(3′b*)), 1.25 (m, CH_3, CH_3^*); ^{13}C NMR (100 MHz, CD_3OD) δ 178.6, 178.5, 76.05, 75.79, 75.77, 75.64, 75.05, 75.00, 72.53, 72.33, 72.18, 71.51, 70.60, 68.93, 46.55, 45.11, 35.99, 35.60, 19.49, 19.23.
9. Honocock, W. S.; Battersby, J. *Anal. Biochem.* **1976**, *71*, 261.