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COMMUNICATION

Chemoenzymatic synthesis of 3-deoxy-3-fluoro-L-fucose and its enzymatic incorporation into glycoconjugates

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The first synthesis of 3-deoxy-3-fluoro-L-fucose is presented, which employs a D- to L-sugar translation strategy, and involves an enzymatic oxidation of 3-deoxy-3-fluoro-L-fucitol. Enzymatic activation (FKP) and glycosylation using an α-1,2 and an α-1,3 fucosyltransferase to obtain two fluorinated trisaccharides demonstrates its potential as a novel versatile chemical probe in glycobiology.

L-Fucose is a 6-deoxy hexose naturally occurring on N- and O-linked glycans and in glycolipids present in a large variety of different organisms.[1, 2] It is the fifth most abundant sugar in mammalian carbohydrates.[3] The presence or absence of fucosylation often crucially determines the structure of biologically relevant antigens such as the histo blood groups and Lewis-type motifs. [2,3] Fucosylated glycans such as the Human Milk Oligosaccharides (HMOs) play an important role in development, and modifications in glycan fucosylation are implicated in immunity, cancer, and many other important biological events.[4-7]

Hence, the importance of this sugar has spurred the development of L-fucose derivatives as probes or inhibitors to investigate and manipulate fucose biosynthesis, and fucosylation of glycans and glycoproteins.[4, 8] Much used inhibitors for enzymatic fucosylation include 2-deoxy-2-fluoro-L-fucose **1** (2DFF, Chart 1)[9,10] and 6,6,6-trifluoro-L-fucose **2**,[11, 12]

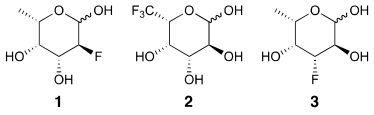
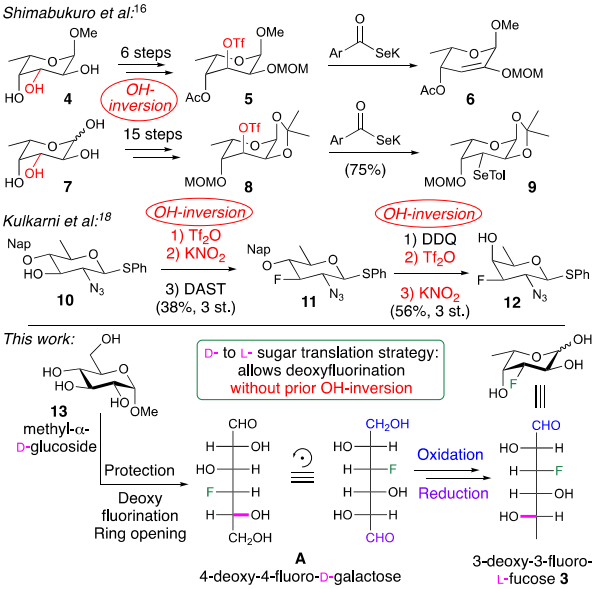


Figure 1. Fluorinated fucose derivatives



Scheme 1. S_N2-reactions at the 6-deoxysugar 3-position.

although 6-fluoro-L-fucose can also be used.[9] 2DFF **1** is commercially available, and a manufacturing process for **2** has been reported.[13] The methyl 4-deoxy-4-fluoro-l-fucose anomers have been used as probes to investigate the DC-SIGN binding epitope, an important receptor of the immune system.[14] Surprisingly, the synthesis of the remaining deoxyfluorinated l-fucose, 3-deoxy-3-fluoro-L-fucose (3DFF) **3**, has not yet been reported.[15]

The synthetic strategy for **3** presents some challenges: the inversion of configuration (in the absence of neighboring group participation) that takes place during deoxyfluorination of

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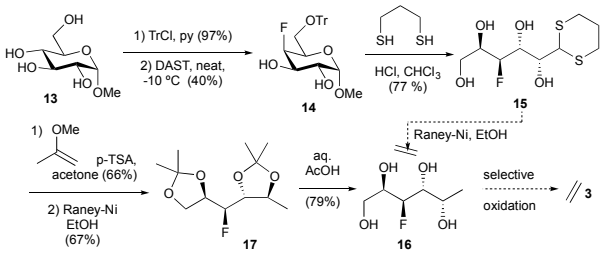
secondary alcohols is a well-known synthetic complication, especially when the required diastereomeric sugar is not available.[16] This is especially so for L-sugars. In such cases, two-step alcohol inversion prior to deoxyfluorination is required, which is often not high-yielding, and all other alcohol groups must be suitably protected. For 3DFF **3** synthesis, a suitably activated 6-deoxy-L-allose intermediate needs to be accessed. Unfortunately, Shimabukuro *et al* have reported that such an S_N2 reaction is very challenging (Scheme 1).[17] Even with an excellent selenide nucleophile, attempted triflate displacement in **5** mainly led to the elimination product **6**. Speculating that the S_N2 reaction may be stereoelectronically disfavoured as predicted by the Richardson-Hough rules,[18] and aiming to hamper the elimination process, a less electron withdrawing 4-OH protecting group and a cyclic protecting group at C1/2 were installed. Now, S_N2 reaction was observed in excellent yield, but at the cost of a very long synthesis of the precursor **8**. In fact, to the best of our knowledge, deoxyfluorination to give 3-fluorinated fuco*pyranose* (or galacto*pyranose*) products has not yet been reported. In their recent syntheses of 3-deoxy3-fluoro-D-fucosamine analogues,[19] Kulkarni *et al.* circumvented the issue by using via 3-OH inversion of the quinovose derivative **10** (equatorial 4-OR group), then deoxyfluorination to give the fluorinated D-quinovosamine derivative **11**, and finally followed by installation of the stereochemistry at C4 by another inversion to obtain the 3-fluorinated D-fucosamine derivative **12**. Hence, this requires multiple inversion steps, and as L-configured quinovose is less available, this route is less suitable for the synthesis of L-**3**.

Sugar epoxides are frequently used for site-selective fluorination. On a pyranose ring, these reactions are usually very regioselective due to the stereo-electronically required axial attack (Furst-Plattner rule).[20] However, in L-fucose the substituent at the 3-position is equatorial, ruling out this option for the synthesis of **3**.

As part of our studies in the use of fluorinated carbohydrates as substrates for enzymatic glycosylations, a practical synthesis of 3DFF **3** was required. The synthetic route in Scheme 1 was deemed unsuitable, given the number of steps involved. In addition, the much reduced nucleophilicity of fluoride compared to selenide was a potential complication that discouraged us from proceeding this way. Driven by the desire to start from a low-cost starting material, and to avoid the requirement of prior inversion of a hydroxyl group in order to arrive at the correct C–F configuration, a synthetic scheme (Scheme 1) was devised that relied on a d- to l-sugar translation. Hence, starting from a suitably protected glucoside **13**, fluorination and ring opening would lead to a 4-deoxy-4-fluoro-d-*galacto*-configured intermediate **A**. Swapping C1 into C6 (D- to L-conversion), followed by respective oxidation/reduction then would lead to the desired fucose derivative **3**. The aldehyde reduction was envisaged via a dithioacetal intermediate. Hence, this strategy is a variation on Barros' d-Gal to L-Fuc synthesis.[21, 22] Other chemical D- to L-sugar syntheses include Okamoto's synthesis of L-Gal from D-Gal,[15] and Fleet's synthesis of L-Glc from D-Glc.[23]

To that end, cheap, commercially available methyl α-D-glucoside **13** (Scheme 3) was selectively protected at the 6-position, upon which treatment with DAST led to selective conversion of the 4-OH group as reported by Card.[24] Without prior anomeric deprotection, the methyl galactoside derivative **14** was converted to the corresponding ring-opened dithioacetal, using an adapted procedure from Redlich and Kölln.[25] The acidic conditions of this reaction also led to the desired trityl hydrolysis, leading to **15**. Unfortunately, all attempts to reduce the dithioacetal group led to the formation of the desired fucitol derivative **16** together with an unidentified and inseparable byproduct.



Scheme 2. Synthesis of 3-deoxy-3-fluoro-L-fucitol, with unsuccessful selective oxidation of the primary alcohol.

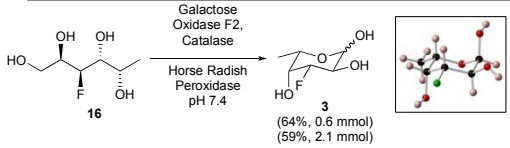
After some experimentation, it was found that acetonide protection of **15** to give **17** allowed for clean dithiane reduction, leading to pure **16**, albeit in moderate yield. Acetonide hydrolysis then afforded pure **16** of which a crystal structure could be obtained. Unfortunately, despite relatively close precedence in the literature,[26-28] all attempts to effect selective oxidation at the primary position gave no result. TEMPO oxidation proved inefficient in converting 3-deoxy-3-fluoro-L-fucitol into **3**, returning the starting material regardless of the conditions used (NaOCl or trichloroisocyanuric acid (TCCA) oxidant). The use of Dess-Martin periodinane did lead to conversion, with NMR analysis indicating a mixture of cyclised products, however, no anomeric signals could be detected indicating overoxidation to lactone derivatives. It was not possible to adjust reaction times to prevent overoxidation. Selective hydrolysis of the terminal acetonide in **17** could also not be achieved.

At this point it was decided to investigate an enzymatic oxidation protocol, as opposed to developing a different protecting group strategy. The Galactose Oxidase (GOase) enzyme was identified as a possible solution. While GOase itself is strictly limited to oxidizing the C6 hydroxyl of non-reducing galactosides and a small number of aromatic primary alcohols, previous engineering efforts have greatly expanded its substrate scope to a large number of primary and secondary alcohols.[29, 30] In this enzymatic alcohol-oxidation protocol, H_2O_2 is produced which is converted back to O_2 using catalase, whilst Horse Radish peroxidase (HRP) is required for GOase activation. Four easily expressed (E. coli) variants of GOase (M_1, M_3, F_2, and $M_{3\text{-}5}$), selected to provide the greatest substrate scope, were tested against **16** for activity (Table 1) using a coupled colorimetric assay that measures the generated H_2O_2. In this

assay HRP utilizes H_2O_2 (generated from the GOase oxidation) to oxidize 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid, ABTS) to a colored product.[31] The variants displayed a wide range of activities: the M_1 variant was unable to oxidize the fluorinated fucitol, with M_3 and $M_{3\text{-}5}$ displaying very low specific activity. However, the F_2 variant provided significant activity useable for scale-up. This F_2 variant was originally generated from mutagenesis of the Arnold Group M_1 variant,[32] and is known to be able to oxidize the primary hydroxyl group of a large panel of saccharides.[29] Interestingly, the specific activity of the F_2-variant towards **16** is higher than its activity towards GlcNAc, which it was engineered towards.[29]

Table 1. GOase variants screened against 25mM 3-Fluoro Fucitol using the HRP-ABTS assay.

GOase variant	M_1	M_3	$M_{3\text{-}5}$	F_2
Specific activity (μmol/min/mg)	-	0.1302 (±0.003)	0.0563 (±0.0004)	2.46 (±0.005)



Scheme 3. Successful synthesis and crystal structure of 3-deoxy-3-fluoro-l-fucose 3

Following a successful analytical scale biotransformation showing full conversion (not shown), we were delighted to observe, after overnight incubation at 25 mM substrate loading, successful conversion of 3-deoxy-3-fluoro-L-fucitol to 3-deoxy-3-fluorofucose **3** in good yield (Scheme 3). Pleasingly, sugar **3** was crystalline, and was found to crystallize as the α-anomer.

In aqueous solution (D_2O), **3** existed as a 1:2 mixture of anomers in favor of the β-anomer, which is essentially the same as the ratio of the nonfluorinated fucose anomers.[33] Analysis of the coupling constants (Figure 1) indicated that both anomers exist in the expected $^1C_4$ conformation: the $^3J_{H1\text{-}H2}$ value was large for the β-anomer and small for the α-anomer, while both anomers showed a large vicinal coupling between H2 and H3. The equatorial position of the fluorine was confirmed by the small $^3J_{F3\text{-}H4}$ values. Interestingly, a large long-range coupling between the equatorial F3 and H1 was observed.

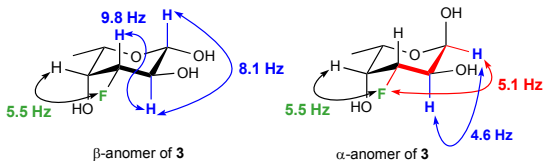
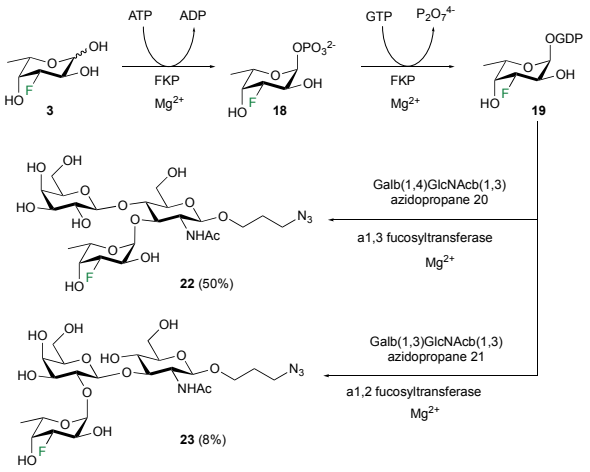


Figure 2. ^{1}H NMR analysis of **3** in D_2O

Next, it was investigated whether the incorporation of 3-deoxy-3-fluorofucose **3** into glycoconjugates containing common motifs found in human biology was possible. Contrary to the well-studied 2-fluorination such as in compound **1**, a known inhibitor of fucosylation in vivo,[9] 3-deoxy fluorination would be expected to have less of an influence on fucosylation reactions and as such would be predicted to allow for fluorine incorporation into glycoconjugates. First the formation of the required fucosyl donor, GDP-3-deoxy-3-fluorofucose **19** (Scheme 4), was achieved using fucokinase/L-fucose-1-P-guanylyltransferase (FKP) from *Bacteroides fragilis*.[34] This bifunctional enzyme converts 3-deoxy-3-fluorofucose **3** into 3-deoxy-3-fluorofucosyl phosphate **18**, and then into GDP-3-deoxy-3-fluorofucose **19** which could be confirmed by mass spectrometry. This demonstrates for the first time that 3-deoxy-3-fluorofucose **3** is a substrate for FKP.

The synthesis of the Lewis x analogue **22** was then attempted from the crude fucosyl donor **19** using an α-1,3-fucosyltransferase from *H. Pylori*.[35] To our delight, the product **22** was isolated in 50% yield after purification showing that 3-deoxy-3-fluorofucose **3** is successfully accepted as substrate. In addition, the synthesis of the type 1 blood group antigen analogue **23** from the fucosyl donor **19** was also successful using a different fucosyltransferase (α-1,2).[36] As a result of limited enzyme availability 20 times less enzyme was used, achieving an 8% yield.



Scheme 4. Enzymatic synthesis of Lewis x analogue **22** and type 1 blood group antigen analogue **23**

In conclusion, we report the first synthesis of 3-deoxy-3-fluoro-L-fucose **3** using a D- to L-sugar conversion strategy, in seven steps starting from a cheap d-glucose starting material. Key aspects of this synthesis are the avoidance of an alcohol inversion operation prior to the deoxyfluorination step, and a final regioselective enzymatic oxidation to the desired 3-deoxy-3-fluoro-L-fucose **3** using a galactose oxidase variant. Contrary to the 2-fluoroisomer **1,** we have shown that **3** can be successfully activated using the kinase FKP to achieve conversion to the corresponding GDP-3-deoxy-3-fluoro-l-fucose **19**, which furthermore is accepted by both an α-1,3- and α-1,2-fucosyltransferase to lead to fluorinated Lewis x **22** and type 1 blood group antigen **23.** This work demonstrates a novel approach to deoxyfluorinated sugar synthesis, as well as the potential of 3-deoxy-3-fluoro-L-fucose **3** as a metabolic probe

that is highly complementary to the widely used 2-fluoro isomer **1**.

Conflicts of interest

"There are no conflicts to declare".

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References

1. M. Schneider, E. Al-Shareffi and R. S. Haltiwanger, *Glycobiology*, 2017, **27**, 601-618.
2. D. J. Becker and J. B. Lowe, *Glycobiology*, 2003, **13**, 41R-53R.
3. D. B. Werz, R. Ranzinger, S. Herget, A. Adibekian, C.-W. von der Lieth and P. H. Seeberger, *ACS Chem. Biol.*, 2007, **2**, 685-691.
4. J. Li, H. C. Hsu, J. D. Mountz and J. G. Allen, *Cell Chem. Biol.*, 2018, **25**, 499-512.
5. L. Veillon, C. Fakih, H. Abou-El-Hassan, F. Kobeissy and Y. Mechref, *Acs Chem. Neurosci.*, 2018, **9**, 51-72.
6. J. J. Listinsky, G. P. Siegal and C. M. Listinsky, *Am. J. Transl. Res.*, 2011, **3**, 292-322.
7. K. Moriwaki and E. Miyoshi, *Trends Glycosci. Glyc.*, 2010, **22**, 239-246.
8. Z. J. Tu, Y. N. Lin and C. H. Lin, *Chem. Soc. Rev.*, 2013, **42**, 4459-4475.
9. C. D. Rillahan, A. Antonopoulos, C. T. Lefort, R. Sonon, P. Azadi, K. Ley, A. Dell, S. M. Haslam and J. C. Paulson, *Nat. Chem. Biol.*, 2012, **8**, 661-668.
10. N. M. Okeley, S. C. Alley, M. E. Anderson, T. E. Boursalian, P. J. Burke, K. M. Emmerton, S. C. Jeffrey, K. Klussman, C.-L. Law, D. Sussman, B. E. Toki, L. Westendorf, W. Zeng, X. Zhang, D. R. Benjamin and P. D. Senter, *Proc. Natl. Acad. Sci. USA*, 2013, **110**, 5404.
11. J. G. Allen, M. Mujacic, M. J. Frohn, A. J. Pickrell, P. Kodama, D. Bagal, T. San Miguel, E. A. Sickmier, S. Osgood, A. Swietlow, V. Li, J. B. Jordan, K.-W. Kim, A.-M. C. Rousseau, Y.-J. Kim, S. Caille, M. Achmatowicz, O. Thiel, C. H. Fotsch, P. Reddy and J. D. McCarter, *ACS Chem. Biol.*, 2016, **11**, 2734-2743.
12. N. C. McKenzie, N. E. Scott, A. John, J. M. White and E. D. Goddard-Borger, *Carbohydr. Res.*, 2018, **465**, 4-9.
13. M. M. Achmatowicz, J. G. Allen, M. M. Bio, M. D. Bartberger, C. J. Borths, J. T. Colyer, R. D. Crockett, T. L. Hwang, J. N. Kolev, S. A. Osgood, S. W. Roberts, A. Swietlow, O. R. Thiel and S. Caille, *J. Org. Chem.*, 2016, **81**, 4736-4743.
14. P. Valverde, S. Delgado, J. D. Martínez, J.-B. Vendeville, J. Malassis, B. Linclau, N.-C. Reichardt, F. J. Cañada, J. Jiménez-Barbero and A. Ardá, *ACS Chem. Biol.*, 2019, **14**, 1660-1671.
15. O. Hindsgaul, D. P. Khare, M. Bach and R. U. Lemieux, *Can. J. Chem.*, 1985, **63**, 2653-2658.
16. W. Song, J. Cai, X. Zou, X. Wang, J. Hu and J. Yin, *Chin. Chem. Lett.*, 2017, **29**, 27-34.
17. J. Shimabukuro, H. Makyio, T. Suzuki, Y. Nishikawa, M. Kawasaki, A. Imamura, H. Ishida, H. Ando, R. Kato and M. Kiso, *Bioorg. Med. Chem.*, 2017, **25**, 1132-1142.
18. K. J. Hale, L. Hough, S. Manaviazar and A. Calabrese, *Org. Lett.*, 2014, **16**, 4838-4841.
19. D. A. Williams, K. Pradhan, A. Paul, I. R. Olin, O. T. Tuck, K. D. Moulton, S. S. Kulkarni and D. H. Dube, *Chemical Science*, 2020, **11**, 1761-1774.
20. A. Furst and P. A. Plattner, *Helv. Chim. Acta*, 1949, **32**, 275-283.
21. R. C. Pinto, M. M. Andrade and M. T. Barros, *Tetrahedron Lett.*, 2012, **53**, 6633-6636.
22. R. Orii, M. Izumi, Y. Kajihara and R. Okamoto, *J. Carbohydr. Chem.*, 2015, **34**, 560-566.
23. R. F. Martinez, Z. Liu, A. F. G. Glawar, A. Yoshihara, K. Izumori, G. W. J. Fleet and S. F. Jenkinson, *Angew. Chem. Int. Ed.*, 2014, **53**, 1160-1162.
24. P. J. Card and G. S. Reddy, *J. Org. Chem.*, 1983, **48**, 4734-4743.
25. O. Kölln and H. Redlich, *Synthesis*, 1995, **1995**, 1376-1382.
26. R. Siedlecka, J. Skarzewski and J. Młochowski, *Tetrahedron Lett.*, 1990, **31**, 2177-2180.
27. R. Polt, D. Sames and J. Chruma, *J. Org. Chem.*, 1999, **64**, 6147-6158.
28. K.-i. Sato, S. Akai, H. Youda, M. Kojima, M. Sakuma, S.-i. Inaba and K. Kurosawa, *Tetrahedron Lett.*, 2005, **46**, 237-243.
29. J. B. Rannes, A. Ioannou, S. C. Willies, G. Grogan, C. Behrens, S. L. Flitsch and N. J. Turner, *J. Am. Chem. Soc.*, 2011, **133**, 8436-8439.
30. F. Escalettes and N. J. Turner, *ChemBioChem*, 2008, **9**, 857-860.
31. A. Toftgaard Pedersen, W. R. Birmingham, G. Rehn, S. J. Charnock, N. J. Turner and J. M. Woodley, *Org. Process Res. Dev.*, 2015, **19**, 1580-1589.
32. L. H. Sun, I. P. Petrounia, M. Yagasaki, G. Bandara and F. H. Arnold, *Protein Eng.*, 2001, **14**, 699-704.
33. S. J. Angyal, *Adv. Carbohydr. Chem. Biochem.*, 1984, **42**, 15-68.
34. W. Wang, T. Hu, P. A. Frantom, T. Zheng, B. Gerwe, D. S. del Amo, S. Garret, R. D. Seidel and P. Wu, *Proc. Natl. Acad. Sci. USA*, 2009, **106**, 16096-16101.
35. D. A. Rasko, G. Wang, M. M. Palcic and D. E. Taylor, *J. Biol. Chem.*, 2000, **275**, 4988-4994.
36. G. Wang, P. G. Boulton, N. W. C. Chan, M. M. Palcic and D. E. Taylor, *Microbiology*, 1999, **145**, 3245-3253.