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

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.\(^5\)\(^-\)\(^7\)

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

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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 although 6-fluoro-\(\alpha\)-fucose can also be used.\(^9\) 2DFF \(1\) is commercially available, and a manufacturing process for \(2\) has been reported.\(^1\)\(^3\) The methyl 4-deoxy-4-fluoro-\(\alpha\)-fucose anomers have been used as probes to investigate the DC-SIGN binding epitope, an important receptor of the immune system.\(^1\)\(^4\) Surprisingly, the synthesis of the remaining deoxyfluorinated l-fucose, 3-deoxy-3-fluoro-\(\alpha\)-fucose \(3\) (3DFF) \(3\), has not yet been reported.\(^1\)\(^5\)

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
secondary alcohols is a well-known synthetic complication, especially when the required diastereomeric sugar is not available. 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 a Sn2 reaction is very challenging (Scheme 1). Even with an excellent selenide nucleophile, attempted triflate displacement in 5 mainly led to the elimination product 6. Speculating that the Sn2 reaction may be stereoelectronically disfavoured as predicted by the Richardson-Hough rules, 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, Sn2 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 fucopyranose (or galactopyranose) products has not yet been reported. In their recent syntheses of 3-deoxy3-fluoro-D-fucosamine analogues, 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). 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 transformation. 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 (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.

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. 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. Without prior anomeric deprotection, the methyl galactoside derivative 14 was converted to the corresponding ring-opened dithioacetal, using an adapted procedure from Redlich and Kolln. 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.

After some experimentation, it was found that acetamide protection of 15 to give 17 allowed for clean dithiane reduction, leading to pure 16, albeit in moderate yield. Acetamide hydrolysis then afforded pure 16 of which a crystal structure could be obtained. Unfortunately, despite relatively close precedence in the literature, 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 acetone 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. In this enzymatic alcohol-oxidation protocol, H2O2 is produced which is converted back to O2 using catalase, whilst Horse Radish peroxidase (HRP) is required for GOase activation. Four easily expressed (E. coli) variants of GOase (M8, M9, F2, and M33), selected to provide the greatest substrate scope, were tested against 16 for activity (Table 1) using a coupled colorimetric assay that measures the generated H2O2. In this
assay HRP utilizes H₂O₂ (generated from the GOase oxidation) to oxidize 2,2'‐azino‐bis(3‐ethylbenzthiazoline‐6‐sulfonic acid, ABTS) to a colored product. The variants displayed a wide range of activities: the M₁ variant was unable to oxidize the fluorinated fucitol, with M₁ and M₃S displaying very low specific activity. However, the F₂ variant provided significant activity useable for scale‐up. This F₂ variant was originally generated from mutagenesis of the Arnold Group M₁ variant, and is known to be able to oxidize the primary hydroxyl group of a large panel of saccharides. Interestingly, the specific activity of the F₂‐variant towards 16 is higher than its activity towards GlcNAc, which it was engineered towards.

Next, it was investigated whether the incorporation of 3‐deoxygenated 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, 3‐deoxygenation 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‐phosphatase (FKP) from Bacteroides fragilis. 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. 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). As a result of limited enzyme availability 20 times less enzyme was used, achieving an 8% yield.

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‐fluorofucose 3 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 (D₂O), 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. Analysis of the coupling constants (Figure 1) indicated that both anomers exist in the expected 1C₆ conformation: the 1JH₁,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 fucose was confirmed by the small 3JF₃,H₄ values. Interestingly, a large long‐range coupling between the equatorial F3 and H1 was observed.

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

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

<table>
<thead>
<tr>
<th>GOase variant</th>
<th>M₁</th>
<th>M₃</th>
<th>M₃S</th>
<th>F₂</th>
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<tr>
<td>Specific activity (µmol/min/mg)</td>
<td>0.1302</td>
<td>0.0563</td>
<td>2.46</td>
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<tr>
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<td>(+0.003)</td>
<td>(+0.0004)</td>
<td>(+0.005)</td>
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Scheme 3. Successful synthesis and crystal structure of 3‐deoxy‐3‐fluorofucose 3

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‐fluorofucose 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‐fluorofucose 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‐fluorofucose 19, which furthermore is accepted by both 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‐fluorofucose 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”.

Acknowledgement

We are grateful to T. Kaminski and M. E. Webb for their contribution in expressing galactosyltransferase enzymes. This project has been funded by the Industrial Biotechnology Catalyst (Innovate UK, BB/SC313536/1, BB/M028983/1) to support the translation, development and commercialization of innovative Industrial Biotechnology processes. BL also thanks the EPSRC (EP/K039466/1), and SF thanks BiLoc, Prozomix Ltd. and Bio-shape Ltd., RCUK (BB/L013762/1; BB/M027791/1; BB/M028836/1) and ERC (788231-Progres-ERC-2017-ADG) for funding. MCG thanks European Research Council (ERC-COG: 648239). JJB thanks the Agencia Estatal de Investigación (Spain) (RTI2018-094751-B-C31 and SEV-2016-0644), and ERC (788143-RECGLYCANMR-ERC-2017-ADG) for funding, and the Ministerio de Educación y Formación Profesional for a FPU fellowship (14/06147) to PV

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