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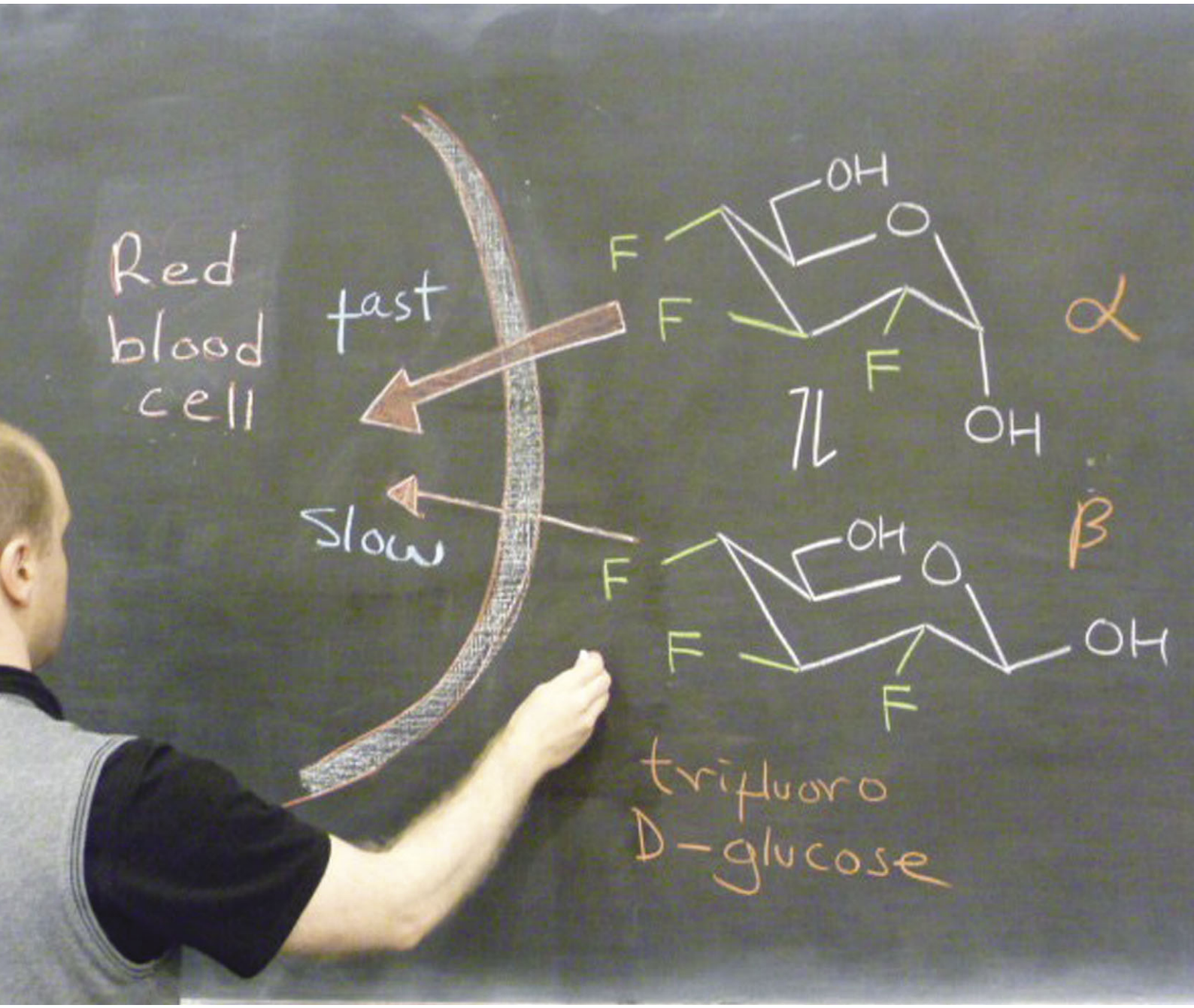

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Fluorosugars: synthesis of the 2,3,4-trideoxy-2,3,4-trifluoro hexose analogues of D-glucose and D-altrose and assessment of their erythrocyte transmembrane transport†‡

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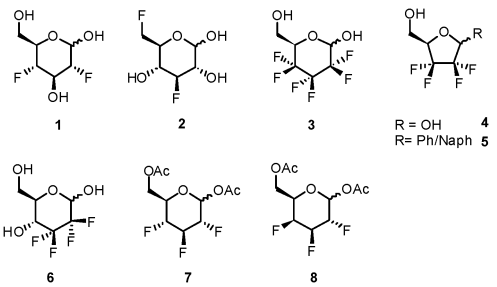
The 2,3,4-trideoxy-2,3,4-trifluoro hexose analogues of D-glucose and D-altrose were prepared and characterised and these novel sugar analogues were explored by 2D-^{19}F-EXSY NMR for their potential to cross erythrocyte (red blood cell) membranes, by comparison with the well known capacity of erythrocytes to transport D-glucose.

Selectively fluorinated carbohydrates have contributed significantly to understanding the role of carbohydrate metabolism and in exploring the mechanism of carbohydrate processing enzymes.[1–3] Typically a single OH group is replaced by fluorine in a carbohydrate to remove the hydrogen bonding donor capacity at that site, but to retain the electronegativity, such that the importance of individual hydrogen bonds for the kinetics of binding, can be assessed in carbohydrate–protein interactions. 2-Fluorocarbohydrates have also proven to be important tools for exploring the mechanism of glycosyl transfer enzymes where the fluorine suppresses the formation of oxocarbenium ion intermediates,[4] and [^{18}F]-2-deoxy-2-fluoro-D-glucose is an important compound clinically, for diagnostic purposes in positron emission tomography (PET).[5] Deoxyfluoro carbohydrates with more than one fluorine have had significantly less currency. There are very few examples in the literature where hexoses with two fluorines replacing secondary alcohols *e.g.* **1** and **2** have been reported.[6,7] There has been some recent interest in much more highly fluorinated sugar analogues. DiMagno *et al.* have prepared hexafluorohexose analogues exemplified by **3**.[8] The high amphiphilic nature of such a carbohydrate gives it unusual physical properties and **3** was shown to be transported across erythrocyte cells an order of magnitude faster than D-glucose, presumably as it had a higher affinity to the relevant transporter protein. Linclau *et al.*[9] have recently developed robust synthesis methodology to prepare highly amphiphilic tetrafluorocarbohydrate analogues exemplified by **4** and **6** and Gouverneur *et al.*[10] have reported the synthesis of tetrafluoro ribose nucleosides such as **5**. In this *Communication* we report the synthesis of hexoses where three adjacent secondary hydroxyls have been replaced by fluorine, with a specific stereochemistry. In this series, Sarda *et al.*[11] reported the synthesis of diacetylated 2,3,4-trideoxy-2,3,4-trifluoro analogues of D-glucose **7** and D-galactose **8**. These acetylated compounds were never hydrolysed to explore the physical or biological properties of the free carbohydrates. We now report the synthesis and characterisation of the 2,3,4-trideoxy-2,3,4-trifluoro hexose analogues of D-glucose **9** and D-altrose **10** as the first examples of such carbohydrate analogues.



The trifluorohexose analogues **9** and **10** were accessed from the key intermediate (*R*)-**11**, which we have recently reported in three steps from 2,3-*O*-(*R*)-isopropylidene-D-glyceraldehyde.[12] Epoxidation of (*R*)-**11** generated a mixture of the two diastereoisomeric epoxides **12a** and **12b** which was treated directly with $Et_3N{\cdot}3HF$ to give a product containing ring-opened isomers as shown in Scheme 1. Diastereoisomers **13** and **14** were separated by chromatography and were then used individually for their conversion to the D-glucose **9** and D-altrose **10** trifluorohexose analogues respectively.

Treatment of **13** with Deoxofluor™ generated the trifluoroacetal **15**. Selective deprotection of the benzyl ether was accomplished by the method of Adinolfi *et al.*[13] to give **16** and then Dess–Martin oxidation gave the unstable α-fluoroaldehyde **17** which was immediately subjected to acetonide cleavage and intramolecular cyclisation, using $SnCl_2$ in CH_2Cl_2[14] to generate the D-glucose analogue **9**. The free sugar **9** is a white crystalline solid and a suitable crystal was subjected to X-ray structure analysis to confirm the relative and absolute stereochemistry which was consistent with configurational inversions for each C–F bond forming reaction. The structure of the resultant β-anomer of **9** is shown in Fig. 1a. In a similar manner difluoroalcohol **14** was treated with Deoxofluor™, debenzylated, oxidised and cyclised as illustrated in Scheme 1 to give the free D-altrose analogue **10**. Likewise a suitable crystal of hexose **10** was subjected to X-ray structure analysis and the resultant

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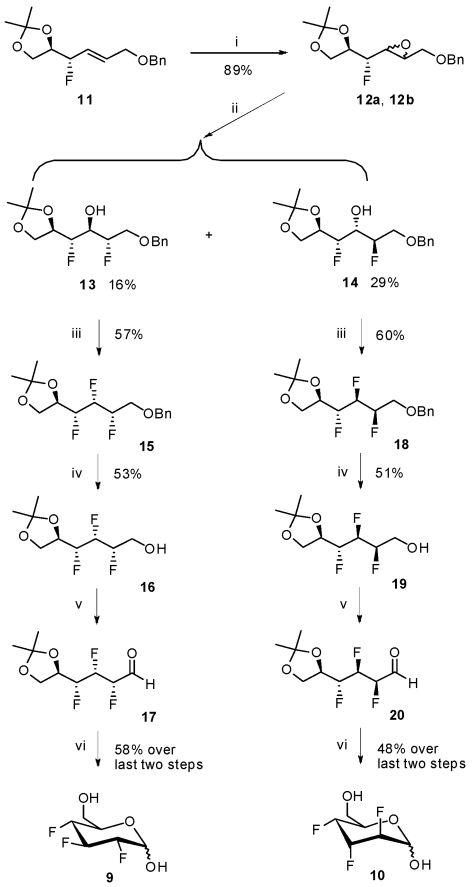
‡ Electronic supplementary information (ESI) available: Experimental procedures and compound characterisation data, X-ray structural data and ^{19}F-EXSY transmembrane studies data. CCDC 775192 and 775193. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c0cc01128b



Scheme 1 Synthesis of D-glucose and D-altrose analogues **9** and **10**. i. *m*CPBA, CH_2Cl_2, RT, 5 d; ii. $Et_3N{\cdot}3HF$, CH_2Cl_2, MW, up to 140 °C, 21 h; iii. Deoxofluor™, CH_2Cl_2, reflux, 14 h; iv. $NaBrO_3/Na_2S_2O_4$, EtOAc, H_2O, RT, 1 h; v. Dess–Martin periodinane, RT, CH_2Cl_2, 40 min; vi. $SnCl_2$, CH_2Cl_2, RT, 1 h.

β-anomer is shown in Fig. 1b. In both cases the crystal packing reveals a special partitioning of the oxygens/hydroxyls from the fluoromethylene residues, an intermolecular pattern presumably dictated by the stronger hydrogen bonding interactions between the oxygen atoms and hydroxyl groups.

The anomeric α : β ratios of both trifluorohexoses **9** and **10** were analysed by $^{19}F\{^1H\}$-NMR spectroscopy in a range of solvents. The individual anomers were assigned by ^1H-NMR, nOe's (see ESI‡). In chloroform the D-glucose analogue **9** favours the α-anomer (~5 : 1), whereas the D-altrose analogue **10** has an approximate 1 : 1 anomeric ratio. It is interesting to note that the minor β-anomer of D-glucose **9** in solution was the anomer that most readily crystallised however the anomeric ratio of the D-glucose analogue **9** tends towards 1 : 1 in increasing polar solvents. Erythrocytes (red blood cells) are recognised to have a transport capability for D-glucose across their cell membranes.[15] The ability of the glucose transporter (Glut1)[15b] to recognise and transport D-glucose analogues can be used as a measure of such analogues to mimic D-glucose. Selectively fluorinated sugars in particular have been explored in erythrocyte trans-membrane studies by exploring the intra- and extra-cellular levels of the sugars using ^{19}F-NMR.[16,17] Previous investigations have shown that 2-deoxy-2-fluoro- and 3-deoxy-3-fluoro-D-glucose analogues are transported at

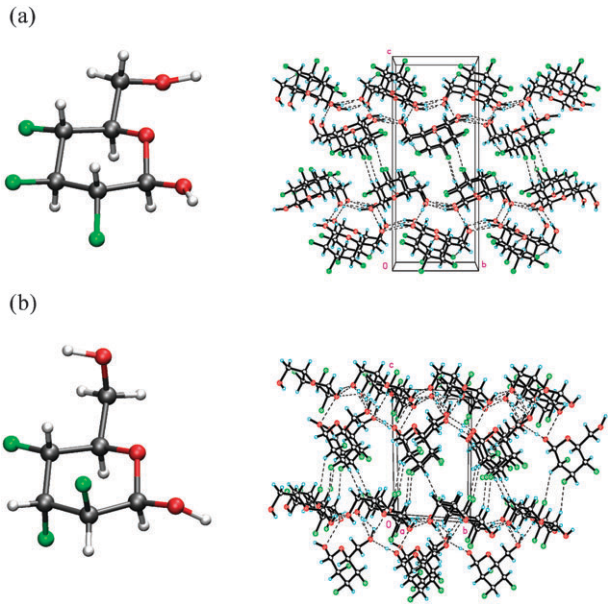


Fig. 1 X-Ray crystal structure and molecular packing of (a) the D-glucose analogue **9** (β-anomer) and (b) the D-altrose analogue **10** (β-anomer). In both cases the crystal packing images highlight a partitioning of the fluorines (green) from the hydrogen bonding interactions between the oxygens/hydroxyls (red) in the solid state.

similar rates to glucose across erythrocyte membranes, but that 4- and 6-deoxy-fluoro analogues transport less well. Also the α-anomer of glucose is more rapidly transported than the β-anomer, and in general this follows for structural analogues. The highly fluorinated hexose analogue **3** has a transport efficiency one order of magnitude faster than 3-deoxy-3-fluoro-D-glucose, and also the α-anomer showing the better performance.[8] The amphiphilic nature of this molecule with both a polar and a fluorous hemisphere may account for this dramatically improved performance. With this background we have explored the transport ability of the trifluoro D-glucose analogue **9** across erythrocyte cell membranes, where the three secondary hydroxyl groups have been replaced in a stereoconservative manner with respect to D-glucose.

For comparison we have also explored the D-altrose analogue **10** which is no longer stereoconservative relative to D-glucose. ^{19}F-NMR and ^{19}F-2D-EXSY NMR experiments were used to study the efflux rate in each case from human erythrocytes. Accordingly cells were suspended in a D_2O solution of the D-hexose analogues in the NMR tube. The resultant ^{19}F-NMR spectra for each analogue could readily identify signals for the intra- and extra-cellular anomers. A typical 2D ^{19}F EXSY-NMR spectrum for **9** is shown in Fig. 2.

The 2D-^{19}F EXSY exchange experiment measures retained nuclear (^{19}F) polarisation from the intra-cellular to the extra-cellular molecular population (efflux rate). The intensity of the cross peaks in this experiment is proportional to the efflux rate constant (k_{ef}). Table 1 reports the resultant rate data for the D-hexose analogues **9** and **10** by comparison with 3-deoxy-3-fluoro-D-glucose. This latter analogue is known to have similar transport ability to D-glucose itself and is a useful reference.[18] Thus we have re-examined 3-deoxy-3-fluoro-D-glucose and find good agreement with the literature in our system.

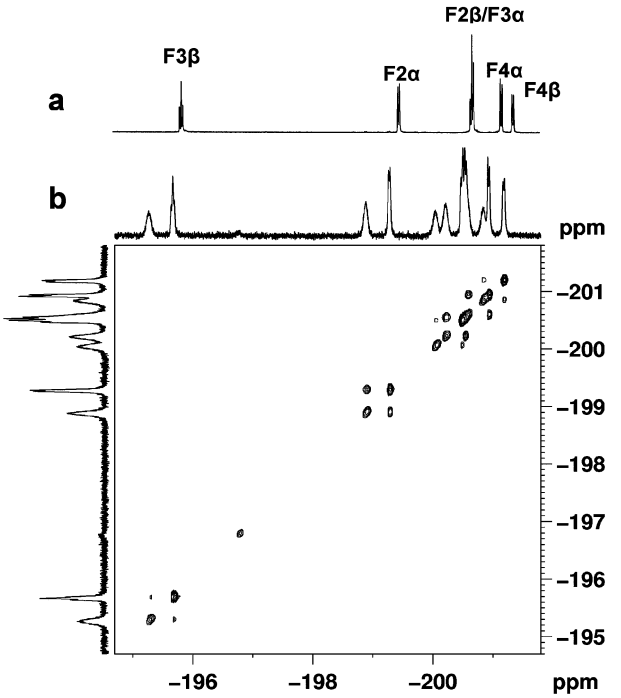


Fig. 2 (a) Control, $^{19}F\{^{1}H\}$NMR spectrum (470.4 MHz) of trifluoro-D-glucose analogue **9** in buffer (123 mM NaCl, 15 mM Tris/HEPES, 5 mM ascorbate) recorded at 310 K shows six resonances corresponding to the α- and β-anomers. (b) 2D ^{19}F EXSY-NMR spectrum (mixing time 500 ms) of trifluoro-D-glucose analogue **9** in the presence of erythrocytes suspended in the buffer at 310 K now shows twelve resonances corresponding to the intra- and extra-cellular populations of the α- and β-anomers. The projection traces show the corresponding $^{19}F\{^{1}H\}$-NMR spectrum. All six resonances of the trifluoro-D-glucose anomers of **9** are accompanied by broad downfield shifted peaks which can be assigned to intracellular trifluoro-D-glucose **9** resonances. The cross-peaks indicate exchange between intra- and extra-cellular pools where the cross-peak intensities are proportional to the exchange rates. Comparison of cross-peaks associated with F2α (−199.3 ppm) and F3β (−195.7 ppm) implies a difference of exchange rate between the α- and β-anomers. There are no cross-peaks corresponding to α/β anomer interconversion, suggesting that mutarotation is negligible w.r.t. the mixing time.

For the D-hexose analogues, both are transported less well, however the D-glucose analogue **9** performs better (~70% efficiency) and there is a clear preference for the α-anomer, with a greater partitioning between α- and β- than any of the other sugars examined, suggesting a level of stereochemical recognition by the Glut1 protein with a similar sense to that of D-glucose. Indeed for the D-altrose analogue **10**, then there is no clear preference and indeed the β-anomer appears to be transported a little faster.

In summary we have presented the first synthesis and structural analyses of trideoxy-trifluoro D-hexose analogues. Transmembrane studies suggest that the Glut1 transmembrane protein can distinguish the D-glucose from the D-altrose analogue, and thus recognise the stereogenicity associated with the C–F bonds, presumably based to some extent on shape and dipolar interactions rather than hydrogen bonding. For the D-glucose analogue **9** the α- and β-anomers are clearly distinguished by the transmembrane protein in favour of the α-anomer, similar to D-glucose itself.

Table 1 Efflux rate constants for **9** and **10** across erythrocyte cell membranes measured by 2D ^{19}F EXSY spectra. The values reported are averages from different ^{19}F resonances and mixing times. Errors are standard deviations

	k_{ef}/s^{-1}	
	α-Anomer	β-Anomer
3-F-D-glucose[a]	1.35 ± 0.32	1.04 ± 0.23
3-F-D-glucose[b]	1.38 ± 0.02	1.01 ± 0.08
9 (D-glucose)	0.97 ± 0.21	0.22 ± 0.07
10 (D-altrose)	0.33 ± 0.07	0.40 ± 0.05

[a] Data from ref. 18. [b] Data reproduced in this work.

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