Structure and Transport Properties of a Novel, Heavily Fluorinated Carbohydrate Analogue

Hong Woo Kim, Paolo Rossi, Richard K. Shoemaker, and Stephen G. DiMagno*

Department of Chemistry
University of Nebraska-Lincoln
Lincoln, Nebraska 68588-0304

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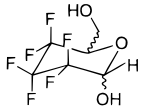
We are interested in heavily fluorinated analogues of biomolecules to probe the relative importance of static and induced dipolar interactions in molecular recognition. Our hypothesis is that induced dipole interactions have a larger effect on the chemical potential of a solute in aqueous solution compared to that of the same molecule in a translationally static environment, such as when it is bound to a protein receptor or active site. If this hypothesis is correct, decreasing the polarizability of a biologically active compound while maintaining its electrostatic charge distribution and shape should lead to enhanced binding to the physiological receptor. We call this strategy enhancing "polar hydrophobicity". Since the C−F bond is among the most polar and least polarizable in organic chemistry (as evinced by the extremely low refractive indices of fluorocarbons),[1] extensive fluorine substitution is a reasonable approach to increase polar hydrophobicity and to improve transport and recognition of biomolecule analogues.

Fluorine substitution is a powerful tool in medicinal and bioorganic chemistry.[2−5] The chemical inertness, relatively small size, and short C−F bond length[1,6] have made C−F substitution attractive for replacement of a number of functional groups, including C−OH, C−H, and C═O. The aggregate size of the C−F group is substantially larger than that of C−H and smaller than that of C−OH. For total spatial extent, the gem-difluoro group should be an adequate substitution for CHOH. Thus, our initial studies have focused on heavily fluorinated carbohydrate analogues, such as 1-hydroxy-5-hydroxymethyl-2,2,3,3,4,4-hexafluorooxane, **1**. These analogues retain the overall shape and



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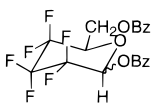

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pseudoquadrupolar charge distribution of the natural compounds but should have diminished polarizability due to the increased fluorocarbon content.

Racemic compound **1** is a somewhat volatile (subl at 57 °C, 0.02 mm), sweet-smelling crystalline material. (Full synthetic procedures for **1** and **2** are given in the Supporting Information.) ^{19}F and ^{1}H NMR studies indicate that the diastereomer ratio (cis/trans or β/α) is solvent dependent, varying from 2:1 in d_6-acetone to 1:1 in aqueous solution. One conformer of each diastereomer was observed at room temperature. The diastereomer assignments for **1** and **2** were made from a Karplus coupling constant analysis[7] for the protons at the anomeric positions ($J_{HF(ax\ proton)} \approx 15$ Hz, $J_{HF(eq\ proton)} \approx 8$ Hz) and confirmed by the isolation and crystal structure determination of anomerically pure **2** (Figure 1).[8]

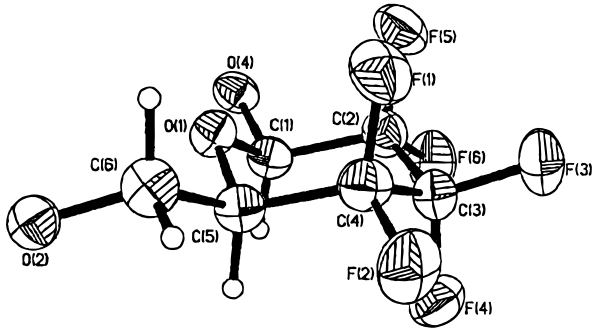

Figure 1. Perspective drawing (50% thermal ellipsoids) from the X-ray crystal structure determination of **2**. The benzoate groups are removed for clarity.

In the core structure of **2** (β-anomer), the pyranose ring adopts the expected chair conformation with the benzoyl protected groups equatorial. The pyranose ring bond lengths in **2** (C−C = 1.52 Å, C1−O1 = 1.407 Å, and C5−O1 = 1.434 Å) are typical of those in carbohydrates (C−C_{ave} = 1.52 Å, C1−O1 = 1.383 Å, and C5−O1 = 1.425 Å for β-D-glucose).[9] A slight expansion in the C2−C3−C4 bond angle (112.1°) notwithstanding, the structure of the carbon skeleton in **2** is virtually superimposable on that of typical β-hexopyranoses.

Human erythrocyte glucose transporter was chosen for initial studies of molecular recognition since it serves as a general model for facilitated diffusion.[10] Studies involving 1-, 2-, 3-, 4-, and 6-deoxyfluoro-D-glucose have been performed, and anomeric preferences for mediated transport through the RBC membrane have been noted.[11−14] 2-Deoxy-2-fluoro-D-glucose (2-DFG) and 3-deoxy-3-fluoro-D-glucose (3-DFG) cross the erythrocyte membrane at rates very similar to that of glucose, while permeabilities for the 4- and 6-substituted derivatives are roughly halved.[11] The α-anomer of each monofluorinated deoxyglucose is transported more rapidly. While fluorine substitution at individual positions on glucose has a relatively small effect on the transport rates, alteration in the configuration of a single hydroxyl group on the ring has a profound effect; for example, galactose is transported >10-fold more slowly than glucose.[15] The transport data from the nonphysiological substrates indicate that the active site is sterically fairly discriminating and that the hydroxyl moieties are most likely interacting with positively charged, hydrogen bond donating, or hydrophobic groups. Thus, this is an ideal receptor to test the polar hydrophobic hypothesis with **1**.

Two ^{19}F NMR experiments (one-dimensional (1D) inversion transfer and two-dimensional exchange spectroscopy (2D-EXSY)) have been used to study the transport of fluorinated compounds across the red blood cell membrane.[11,13] These techniques exploit the small ^{19}F chemical shift differences obtained for compounds in the intra- and extracellular environments. For compound **1**, *J*-coupling among the fluorine nuclei on the pyranose ring results

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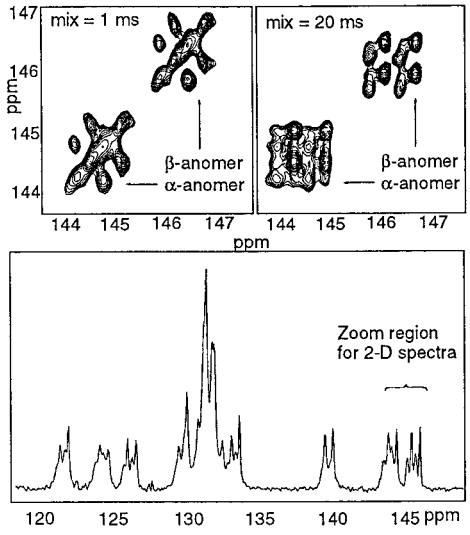

Figure 2. Sample 1D and 2D exchange data showing the mixing time dependence of the transport of **1**, as described in the text. Exchange is evinced by the cross-peaks in the 2D plots at 20 ms, particularly for the α-anomer.

in overlapping resonances from the intra- and extracellular compound, necessitating a two-dimensional experiment (Figure 2).

NMR samples were prepared as described previously.[11,16] The one-dimensional ^{19}F spectrum (Figure 2) shows that the α- and *β*-anomers are present in a 1:1 ratio and that each fluorine doublet is split into two signals. For each signal, the downfield, broader doublet is due to the intracellular analogue, as verified by gathering similar spectra at several different hematocrits. Close inspection of the signals at 145 ppm (α-anomer) and 147 ppm (*β*-anomer) reveals that the α-anomer partitions into the cell to a lesser extent than the *β*-anomer $\{(k_{in}/k_{ef})_\alpha < (k_{in}/k_{ef})_\beta$ where k_{in} and k_{ef} represent the influx and efflux transport rate constants, respectively$\}$. Curve fitting of the signals at 145 and 147 ppm yielded $([\alpha]_{in}/[\alpha]_{ex}) = 0.55$ and $([\beta]_{in}/[\beta]_{ex}) = 0.71$ for the ratios of the intracellular and extracellular concentrations of **1**.

Two-dimensional exchange spectroscopy was employed to determine the absolute magnitudes of the transmembrane transport rate constants.[17] Mixing times of 1, 20, 35, and 50 ms were employed, and each experiment was duplicated. Sample deterioration was minimal in sealed tubes, and repeated runs gave indistinguishable data. There were no extraneous signals in the ^{19}F NMR spectrum at the conclusion of the experiments, indicating that the substrate was not metabolized. Data from runs conducted at two different mixing times are given (using identical plot parameters) in Figure 2. The plot shows significant cross-peak intensities even at very short mixing times (20 ms), indicating the membrane is extremely permeable to **1**. Furthermore, the α-anomer is transported at a rate that is significantly greater than that of the *β*-anomer, consistent with earlier results from selectively fluorinated carbohydrates.

Exchange rates for transmembrane transport were determined by integrating slices through the 2D spectra and fitting the cross-peak intensity (normalized to the diagonal peak intensity) (I_X/I_D) to eq 1

$$(I_X/I_D) = A + (1 - \exp(-T_{mix}/k_{exch}))/(1 + \exp(-T_{mix}/k_{exch})) \quad (1)$$

where the adjustable parameters were the offset (A) and the total exchange rate constant (k_{exch}).[18] T_{mix} was the mixing time parameter in the individual 2D-EXSY experiments. By normalizing the cross-peak to the diagonal peak, the contribution from relaxation effects is canceled out. The total exchange rate constants obtained from this analysis were 54 s^{-1} for the α-anomer, and 15 s^{-1} for the *β*-anomer. Individual efflux (k_{ef}) rate constants were extracted from k_{exch} after correcting for the differences in intra- and extracellular volumes, giving $k_{ef} = 22.2$ s^{-1} for the α-anomer and $k_{ef} = 7.6$ s^{-1} for the *β*-anomer. Efflux permeabilities (P_{ef}) were calculated for the individual anomers using eq 2

$$P_{ef} = (V_i/A)k_{ef} = (\mathrm{MCV}f_w/A_{cell})k_{ef} \quad (2)$$

where V_i is the intracellular volume, A is total membrane area, MCV is the mean cellular volume (85 fL for RBCs in isotonic solution), $A_{cell} = 1.43 \times 10^{-6}$ cm^2 is the surface area per cell, and $f_w = 0.717$ is the fraction of the intracellular volume which is accessible to solutes.[19] A comparison of the permeability measured for **1** ($P_{ef\alpha} = 9.5 \times 10^{-4}$ cm/s and $P_{ef\beta} = 3.2 \times 10^{-4}$ cm/s) with those determined for 3-DFG ($P_{ef\alpha} = 0.58 \times 10^{-4}$ cm/s and $P_{ef\beta} = 0.44 \times 10^{-4}$ cm/s) shows that the heavily fluorinated analogue crosses the RBC membrane at an approximately 10-fold higher rate.

Finally, it should be noted that the introduction of fluorine into organic compounds generally increases their lipophilicity (π),[20,21] a factor that is often cited for their increased transport and bioavailability.[22] Three experiments were performed to ascertain whether the transport of **1** was facilitated by glucose transporter or resulted from increased rates of simple transmembrane diffusion. First, the 2D-EXSY experiment was carried out at 25 °C. No exchange was observed under these conditions, indicating that the exchange rates had decreased by at least a factor of 100. For simple diffusion, the exchange rate at 25 °C should be 0.96 times that at 37 °C, according to the Stokes−Einstein equation.[23] Second, an RBC suspension containing **1** was incubated (2 h at 37 °C) with phloretin, a known inhibitor of glucose efflux,[24] before the 2D-EXSY experiment. Transmembrane exchange was reduced by a factor of 3 after this treatment. Third, transport of **1** was observed to decrease in solutions containing added glucose (>100 mM). These experiments indicate that the increased permeability of **1** is consistent with mediated diffusion and enhanced specific binding to the transport protein.

The data reported here indicate that increasing the polar hydrophobicity of judiciously chosen substrates may be a useful strategy to improve biological molecular recognition. It remains to be seen whether this strategy can be broadly applied to diverse biomolecules. However, given the large number of pharmaceutically relevant compounds that contain terminal hexopyranose moieties, there is ample opportunity to explore the effects of appending heavily fluorinated carbohydrate analogues in well-studied systems, provided that the requisite synthetic methodology is developed.

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Supporting Information Available: Experimental procedures and characterization data for **1** and **2**, detailed description of the 2D NMR experiment and data analysis, and crystallographic data for **2** (18 pages, print/PDF). See any current masthead page for ordering information and Web access instructions.

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(16) NMR samples containing suspended human red blood cells (RBCs) (final hematocrit ~0.5) and **1** (17.6 mmol) in aqueous buffer (123 mM NaCl, 15 mM Tris-HEPES, and 5 mM ascorbic acid) were treated with carbon monoxide and placed in 5-mm tubes topped with resealable Teflon valves. (A D_2O std was contained within an internal capillary.) Samples were equilibrated at 37 °C for 15 min before data acquisition, and the spinner air was turned off to prevent centrifugation.

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