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Synthesis and Testing of 2-Deoxy-2,2-Dihaloglycosides as Mechanism-Based Inhibitors of α-Glycosidases

Ran Zhang,[†] John D. McCarter,[†] Curtis Braun,[†] Wai Yeung,[†] Gary D. Brayer,[‡] and Stephen G. Withers*,[†,‡]

Department of Chemistry, University of British Columbia, 2036 Main Mall, Vancouver, British Columbia, Canada V6T 1Z1, and Department of Biochemistry and Molecular Biology, University of British Columbia, Vancouver, British Columbia, Canada, V6T 1Z3

withers@chem.ubc.ca

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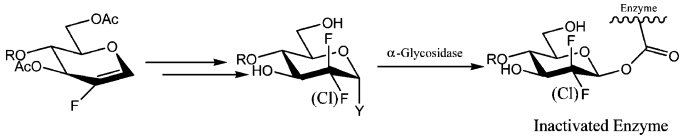

The synthesis of a series of 2-deoxy-2,2-dihaloglycosyl halides as potential α-glycosidase inactivators has been achieved *via* the halogenation of protected 2-fluoroglycal precursors. Direct chlorination of per-*O*-acetylated 2-fluoro-D-glucal and 2-fluoromaltal followed by basic deprotection yielded the corresponding 2-chloro-2-deoxy-2-fluoroglycosyl chlorides. Reaction of the per-*O*-acetylated 2-fluoroglycals with acetyl hypofluorite or Selectfluor yielded the 2-deoxy-2,2-difluoroglycosyl derivatives, which were converted to their α-chlorides using thionyl chloride and deprotected under basic conditions. Trinitrophenyl glycosides of the 2-deoxy-2,2-difluoro mono- and disaccharides were synthesized by arylation of the hemiacetals with picryl fluoride, then deprotected with HCl in methanol. All three monosaccharide derivatives caused active site-directed, time-dependent inactivation of yeast α-glucosidase *via* the trapping of covalent glycosyl−enzyme intermediates, and kinetic parameters for inactivation by each compound were determined. Surprisingly neither of the 2-deoxy-2,2-dihalomaltosyl chlorides caused time-dependent inactivation of human pancreatic α-amylase, despite the fact that the trinitrophenyl 2-deoxy-2,2-difluoromaltoside functioned in that mode. The trinitrophenyl glycosides appear to be approximately 1000-fold more reactive than the corresponding chlorides in the enzyme active sites.

Introduction

Glycosidases are involved in a range of important biological processes, and potent inhibitors of these enzymes have shown promise as therapeutics for diseases such as diabetes, obesity, lysosomal storage diseases and influenza.[1] Indeed several glycosidase inhibitors are already used clinically for treating some of these diseases.[2−3] While interest has generally focused on reversible inhibitors of glycosidases, irreversible inhibitors have proved to be extremely important in providing structural and mechanistic insights[4−5] that not only are of academic interest but also provide guidance for the rational design and optimization of reversible inhibitors. Indeed, given the importance of such irreversible-inhibitor drugs as penicillin and aspirin, mechanism-based inhibitors of glycosidases and other enzymes may also prove valuable, despite industry prejudices against such approaches.

The vast majority of glycosidases employ one of two fundamental mechanisms, first described by Koshland,[6] that are based on the stereochemical outcomes of the hydrolysis reactions they catalyze.[7] Inverting enzymes use a single displacement mechanism with acid/base catalysis and oxocarbenium ion-like transition states. Retaining glycosidases generally use a double displacement mechanism (Figure 1) in which the first step involves an acid-catalyzed nucleophilic displacement of the aglycone by a carboxylate residue at the active site (glycosylation step). In a second step the covalent glycosyl−enzyme

[†] Department of Chemistry, University of British Columbia.
[‡] Department of Biochemistry and Molecular Biology, University of British Columbia.

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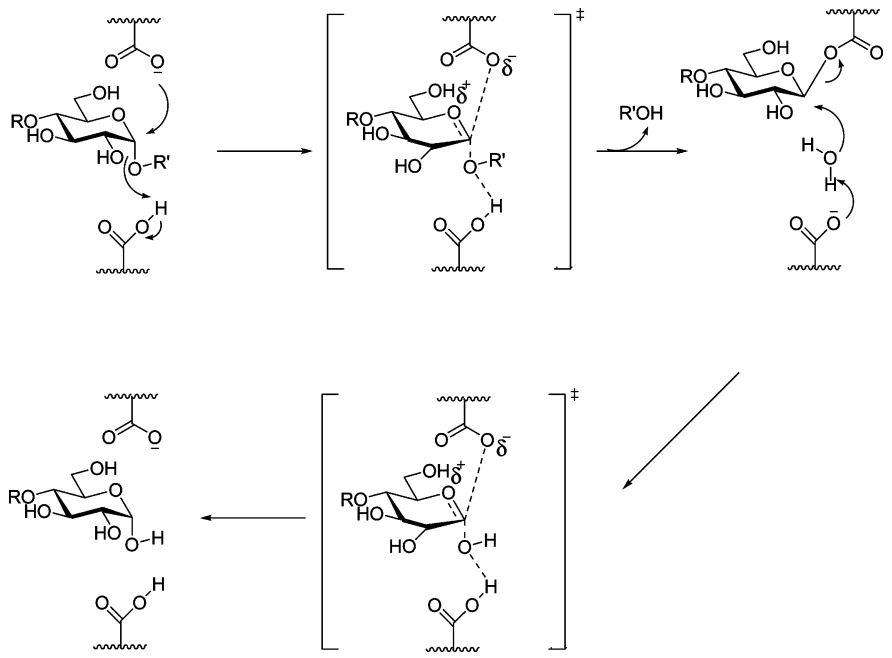

FIGURE 1. Double displacement mechanism of retaining α-glycosidases.

intermediate is then hydrolyzed, with general base catalysis provided by the same group that earlier served as the acid catalyst (deglycosylation step). Both steps involve oxocarbenium ion-like transition states.

2-Deoxy-2-fluoroglycosides with activated leaving groups have proved to be a very useful class of mechanism-based inhibitors for retaining *β*-glycosidases that function *via* the formation of a relatively stable α-linked 2-deoxy-2-fluoroglycosyl−enzyme intermediate.[8−9] The presence of a fluorine at C-2 destabilizes the oxocarbenium ion-like transition states both inductively and via the removal of key transition state-stabilizing interactions that are normally present at C-2. As a consequence both the formation and the hydrolysis of the glycosyl−enzyme intermediate are slowed down. However, the incorporation of a very good leaving group ensures that the intermediate is kinetically accessible, thus that it accumulates. This approach has been applied to many retaining *β*-glycosidases and the resultant trapped intermediates have been characterized by NMR spectroscopy, MS, and X-ray crystallography, thereby providing great insights into each enzyme's mechanism.[10−15]

While this strategy has been highly successful with a range of retaining *β*-glycosidases, it has not been generally successful with α-glycosidases.[9,16] In these latter cases the deglycosylation step tends to be fast relative to the glycosylation step and the effects of fluorine substitution at the C-2 position tend to be proportionally greater on the glycosylation step than the deglycosylation step. Consequently the 2-deoxy-2-fluoro-α-glycosides tend to function as slow substrates, for which the glycosylation step is rate-limiting. Two solutions to this problem have been devised. In one approach a fluorine introduced at C-5 seems to inductively destabilize the deglycosylation step for α-glycosidases more than the glycosylation step, thus when C-5 fluorination is coupled with the incorporation of a good leaving group (fluoride) at C-1, inactivation may be achieved.[17] Unfortunately, synthetic approaches to the disaccharide derivatives that would be needed for amylases, for which tight-binding inhibitors might serve as novel anti-diabetes drugs, have not been developed since selective fluorination at C-5 and not C-5′ has not proved possible, and reaction yields are insufficient to effect successful fluorination at *both* sites. The other approach has been to introduce two fluorines at C-2, thereby slowing down both steps enormously.[18−19] This requires the incorporation of a yet better leaving group at the anomeric center in order to allow the glycosylation step to proceed. To date this has been achieved by the use of a 2,4,6-trinitrophenyl (picryl) group (TNP). The presence of the two fluorines at C-2 ensures that the compound has the necessary inherent solvolytic stability to be deprotected and survive in aqueous solution. Three such compounds, all with two fluorines at C-2 and a TNP group at the anomeric center, have been reported previously[20−21] and all act as excellent, mechanism-based inactivators for their corre-

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sponding enzymes. Indeed the 2,2-difluorogalactosyl derivative has been used to identify the catalytic nucleophile in an α-galactosidase from family 27.[21] However, details of the synthesis of this class of compounds have not been reported.

While the use of *gem*-difluoro substitution has a certain esthetic appeal, and minimizes the chances of steric conflicts, it is not clear that both halogens need to be fluorine. A chlorine substituent would provide a similar inductive effect at C-2, and while larger than fluorine, it is still of a comparable size to the hydroxyl group originally at that position. Replacement by chlorine would also provide certain advantages in terms of synthesis since the introduction of chlorine is generally simpler than introduction of fluorine. Yet more importantly, through the use of Cl_2 as chlorinating agent it may be possible to also, simultaneously, introduce an excellent leaving group at C-1 in the form of a chloride. We have found that sugars bearing anomeric chlorides have generally proved to be too labile to be deprotected, even in the presence of a fluorine substituent at C-2, vividly illustrating the relatively high reactivity achievable with such an aglycone. However, it seems probable that, when accompanied by dihalo substitution at C-2, such deprotected sugars may prove sufficiently stable to survive aqueous conditions, but still reactive enough to undergo the first step of a retaining glycosidase mechanism and thereby trap the intermediate. An additional potential advantage is that, by introducing halides of different sizes at C-2 it may be possible to generate inhibitors that are selective for α-glucosidases over α-mannosidases, by incorporating an equatorial chlorine and an axial fluorine, or *vice versa* to produce selective α-mannosidase inhibitors.

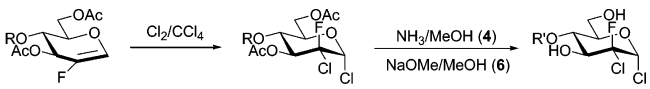
There are three general strategies by which sugars containing geminal fluorine substituents have been synthesized, though in most of the cases published the products have not additionally included a highly reactive leaving group. The most common approach is to selectively oxidize the hydroxyl group at the site in question and then react the ketone with DAST to introduce the *gem*-difluoro functionality.[22−25] However, the success of this reaction is highly dependent on the structure of the substrate, particularly with respect to the identities and stereochemistry of neighboring groups.[26−27] In some cases yields of over 80% were obtained while in other cases, moderate yields or even no detectable yields were observed due to competing side reactions such as migrations and eliminations. A second approach involves a Reformatsky reaction in which acyclic *gem*-difluoro precursors such as ethyl bromotrifluoroacetate or bromodifluoromethyl phenyl acetylene are coupled with a correctly configured aldehyde, then the product cyclized to complete the synthesis of the *gem*-difluoro-substituted sugar ring.[28−29] While this approach was successful for the assembly of 2-deoxy-2,2-difluororibose, it requires maintenance of exquisite control over the stereochemistry of the ring hydroxyls and would be especially challenging for the synthesis of disaccharides. The third approach, which was the one we followed, involves the initial synthesis of a 2-fluoroglycal, which is then reacted with an electrophilic fluorine source such as Selectfluor, F_2, acetylhypofluorite or trifluoroxymethane.[25,30−31] The *gem*-difluoro sugars can be obtained in respectable yields in this manner. This route is also highly compatible with the synthesis of 2-deoxy-2-chloro-2-fluoro sugars, since the same fluoroglycal intermediate can be reacted with electrophilic chlorine sources. Indeed, by use of Cl_2 as the chlorinating agent it should be possible to introduce chlorines at both the C-2 and C-1, with the latter potentially serving as a suitably reactive leaving group. The initial choice of a trinitrophenyl leaving group at C-1 of these *gem*-dihalo sugars was based upon its substantially better leaving group ability (pK_a of picric acid = 0.4) than that of the 2,4-dinitrophenyl leaving group (pK_a = 4.0) used with the monofluorosugar inhibitors, as needed to offset the rate retardation in the glycosylation step effected by the two adjacent halogens.[6] It was also advantageous synthetically since it could be installed by reaction of the sugar hemi-acetal with picryl fluoride, thereby avoiding potentially challenging displacement reactions at a highly deactivated anomeric center using a very weak phenol nucleophile.

This manuscript describes the synthesis of several 2-deoxy-2,2-dihaloglycosyl derivatives in both a monosaccharide and a disaccharide series, with anomeric leaving groups of either chloride or 2,4,6-trinitrophenolate. It further describes the kinetic evaluation of these reagents as mechanism-based inhibitors of both the yeast α-glucosidase and the human pancreatic α-amylase.

Results and Discussions

I. Synthesis of 2-Chloro-2-deoxy-2-fluoro-α-D-glucosyl chloride (1) and 2-chloro-2-deoxy-2-fluoro-α-maltosyl chloride (2) (Scheme 1). 3,4,6-Tri-*O*-acetyl-2-fluoro-D-glucal, **3**, prepared essentially as described previously from commercially available D-glucal,[31] was dissolved in cooled carbon tetrachloride (−23 °C) and subjected to chlorination with chlorine gas.[32−34] Early studies on the addition of chlorine to the double bond of tri-*O*-acetyl-D-glucal had shown that the stereochemical outcome for this addition depends on the solvent polarity, with nonpolar solvents favoring *syn* addition while polar solvents favor *anti* addition.[34] Indeed, the major product isolated in this case was that of *syn* addition from the α-face, namely 3,4,6-tri-*O*-acetyl-2-chloro-2-deoxy-2-fluoro-α-D-glucopyranosyl chloride, **4**. This compound was readily deprotected using ammonia in methanol to yield the desired target **1**, which was purified by silica gel

SCHEME 1



3: R = Ac
5: R = $(OAc)_4$Glc-α
4: R = Ac
6: R = $(OAc)_4$Glc-α
1: R' = H
2: R' = Glc-α

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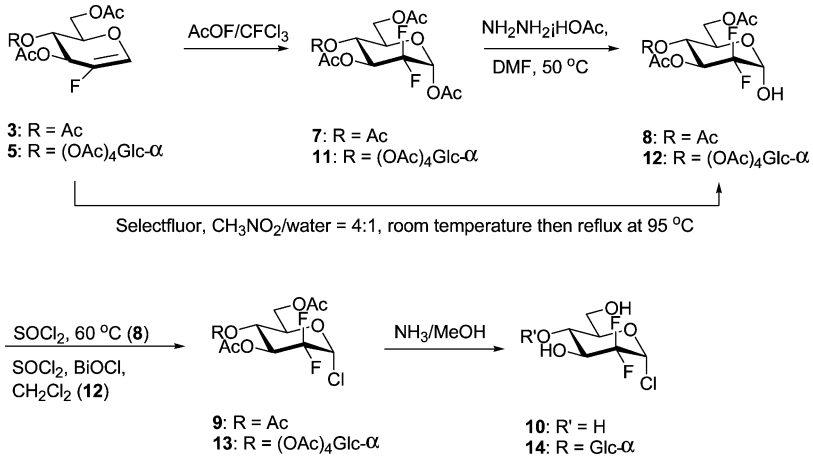
SCHEME 2



chromatography. Likewise, hexa-*O*-acetyl-2-fluoromaltal (**5**) (synthesis available in the Supporting Information) was similarly reacted with chlorine gas dissolved in cold carbon tetrachloride then warmed to room temperature, yielding 3,6-di-*O*-acetyl-4-*O*-[2′,3′,4′,6′-tetra-*O*-acetyl-α-(1,4)-D-glucosyl]-2-chloro-2-deoxy-2-fluoro-α-D-glucopyranosyl chloride (**6**) as the major product in 49% yield. After purification by flash chromatography and characterization, the disaccharide derivative **6** was de-*O*-acetylated in 82% yield using sodium methoxide in methanol. The *syn* addition stereochemistry in each case was clearly shown by analysis of the ^{1}H and ^{19}F NMR spectra of the addition products. Large $J_{3,F}$ coupling constants of 22.8 and 23.3 Hz clearly indicated an axial fluorine at C-2 with a trans-diaxial coupling to H-3. In addition, the presence of relatively small $J_{1,F}$ coupling constants of 6.0 and 6.1 Hz clearly indicated the absence of a trans-diaxial coupling in that case, thus the presence of an equatorial proton at C-1, and therefore of an axial anomeric chlorine.

II. Synthesis of 2-Deoxy-2,2-difluoro-α-D-*arabino*hexopyranosyl chloride 10 and 2-deoxy-2,2-difluoro-4-*O*-[α-(1,4)-D-glucopyranosyl]-α-D-*arabino*hexopyranosyl chloride 14 (Scheme 2). 3,4,6-Tri-*O*-acetyl-2-fluoro-D-glucal **3** was subjected to electrophilic fluorination with acetyl hypofluorite, as described previously[31] to afford 1,3,4,6-tetra-*O*-acetyl-2-deoxy-2,2-difluoro-α-D-arabinohexopyranose (**7**) in a good yield (76%). All attempts to convert this directly to the α-chloride by treatment with HCl proved unsuccessful, lending support to the notion that the presence of geminal fluorine substituents at C-2 would severely slow down the displacement chemistry at the anomeric center. A two-step chlorination process was therefore used in which hydrazine acetate was first used to selectively deprotect the anomeric acetate, yielding the α-hemiacetal **8**. Reaction of this product with thionyl chloride at 60 °C for 3 days provided a reasonable yield (62%) of 3,4,6-tri-*O*-acetyl-2-deoxy-2,2-difluoro-α-D-arabinohexopyranosyl chloride (**9**). The formation of the α-chloride is most likely a consequence of an initial *β*-chloride formation from displacement of the α-sulfinyl chloride, followed by equilibration to the thermodynamically favored α-chloride driven by the excess chloride in solution. This anomeric stereochemistry is clearly evidenced by the two small $J_{F,1}$ coupling constants of 4.8 and 2.0 Hz measured. An axial anomeric proton would exhibit one large trans-diaxial coupling and one small. Alternatively, synthesis of **8** can be achieved by directly reacting 3,4,6-tri-*O*-acetyl-2-fluoro-D-glucal (**3**) with Selectfluor in a nitromethane/water mixed solvent.[35] This route employs a much more stable and safer fluorinating agent, but in this case suffered from a lower yield (around 20%) in the fluorination step. However, when applied to the fluorination of the 2-fluoromaltal, an excellent yield (>80%) was obtained. Deprotection of **9** was simply achieved using ammonia in methanol.

Similar chemistry was employed in the conversion of hexa-*O*-acetyl-2-fluoromaltal (**5**) to the 2-deoxy-2,2-difluoro-4-*O*-[α-(1,4)-D-glucopyranosyl]-α-D-arabinohexopyranosyl chloride (**14**) except that thionyl chloride could not be employed for the chlorination step since the acidic conditions resulted in cleavage of the interglycosidic linkage. Simple lowering of the reaction temperature was not useful since under these conditions no chlorination occurred. After unsuccessfully experimenting with a range of chlorination conditions, including the use of triphenylphosphine with *N*-chlorosuccinimide,[36] we were delighted to discover that the addition of bismuth(III) oxychloride (BiOCl) to thionyl chloride allows the chlorination reaction to proceed at room temperature, albeit in low yield.[37] BiOCl is thought to act as a pro-catalyst for $BiCl_3$, which is difficult to handle, but which itself acts as an efficient Lewis acid catalyst for a range of reactions.[38−39]

III. Synthesis of 2,4,6-Trinitrophenyl 2-deoxy-2,2-difluoro-α-D-arabinohexopyranoside (16) and 2,4,6-trinitrophenyl-2-deoxy-2,2-difluoro-4-*O*-[α-(1,4)-D-glucopyranosyl]-α-D-arabinohexopyranoside 18. (Scheme 3). Reaction of 3,4,6-tri-*O*-acetyl-2-deoxy-2,2-difluoro-α-D-arabinopyranose (**8**) with 1-fluoro-2,4,6-trinitrobenzene in dichloromethane in the presence of the hindered base 2,6-di-*tert*-butylpyridine for 10 days at room temperature in the dark under nitrogen provided a satisfactory yield (51%) of the desired product **15**, which was deprotected using acetyl chloride in methanol. Conversion of the maltose derivative **12** was achieved in an excellent yield (91%) under identical conditions, and the protected disaccharide was also deprotected using acetyl chloride in methanol to yield the final compound **18** in 71% yield after purification by flash

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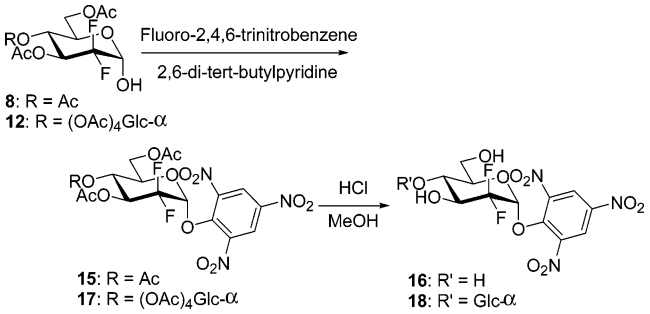
SCHEME 3



chromatography. In both cases, the α-anomeric configurations of the products are clearly indicated by the small (3.6 and 5.3 Hz) $J_{1,\ F2(ax)}$ coupling constants. A β-configured product would have exhibited a large, trans-diaxial coupling constant $J_{1,\ F2(ax)}$ with the axial anomeric proton.

Kinetic Studies. Yeast α-glucosidase is a well behaved α-glycosidase whose inhibition and inactivation has been studied extensively previously, thus was chosen as the model enzyme for the testing of the monosaccharide-based inactivators.[20,40−41] Indeed, the time-dependent inactivation of yeast α-glucosidase by compound **16**, 2,4,6-trinitrophenyl-2-deoxy-2,2-difluoro-α-arabinohexopyranoside, had been studied previously[20] and yielded a second-order rate constant for inactivation of $k_i/K_i = 0.25\ \text{min}^{-1}\ \text{mM}^{-1}$. Similarly, both the 2-chloro-2-fluoro-α-glycosyl chloride, **1**, and the 2,2-difluoro-α-glycosyl chloride, **10**, caused time-dependent inactivation of yeast α-glucosidase, as discussed below.

Pseudo-first-order kinetics of inactivation were seen at each concentration of **1**, allowing the determination of a series of rate constants for inactivation (k_{obs}) at each inactivator concentration (Figure 2). A replot of these inactivation rate constants versus inactivator concentration allowed the determination of inactivation parameters of $k_i = 0.25 \pm 0.06\ \text{min}^{-1}$ and $K_i = 47 \pm 19$ mM, thus a second-order rate constant for inactivation of $k_i/K_i = (5.3 \pm 3.4) \times 10^{-3}\ \text{min}^{-1}\ \text{mM}^{-1}$. Active site-directed inactivation is demonstrated by the protection against inactivation afforded by the known competitive inhibitor 1-deoxynojirimycin (63 μM; $K_i = 25\ \mu$M[42]), which reduced the k_{obs} at 11.3 mM of compound **1** from 0.056 min^{-1} to 0.020 min^{-1}. No reactivation of inactivated enzyme that had been dialyzed to remove excess inactivator was seen upon incubation at 37 °C in buffer, even after incubation for 10 days.

Similarly, time-dependent inactivation of yeast α-glucosidase was observed upon incubation with the 2-deoxy-2,2-difluoro-α-D-arabinohexopyranosyl chloride **10** (data not shown). Individual inactivation parameters of $K_i = 9.7 \pm 2.7$ mM and $k_i = (8.8 \pm 1.0) \times 10^{-4}\ \text{min}^{-1}$ were determined, yielding a second-order rate constant for inactivation of $(9.1 \pm 3.3) \times 10^{-5}\ \text{min}^{-1}\ \text{mM}^{-1}$. Protection against inactivation by 200 μM 1-deoxynojirimycin reduced k_{obs} from $3.7 \times 10^{-4}\ \text{min}^{-1}$ to $9.9 \times 10^{-5}\ \text{min}^{-1}$ at 11 mM of compound **10**, showing that inactivation is active-site directed. Once again no reactivation was observed after incubation of the inactivated enzyme, which had been freed of excess inactivator in buffer over 3 days at 37 °C. This is consistent with formation of an extremely stable glycosyl−enzyme intermediate.

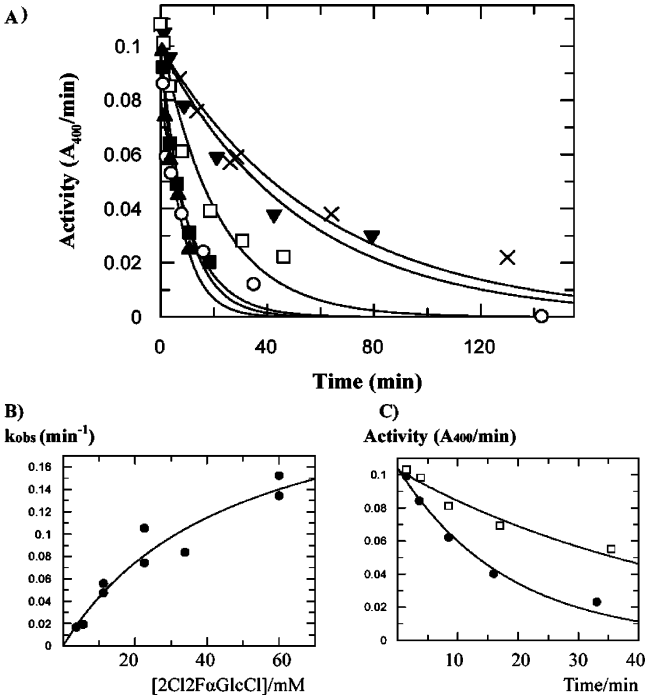


FIGURE 2. Inactivation of yeast α-glucosidase by 2-chloro-2-deoxy-2-fluoro-α-D-glucosyl chloride (**1**). A) Plot of residual activity versus time at the indicated inhibitor concentrations: 3.78 mM (×), 5.67 mM (▼), 11.34 mM (□), 22.7 mM (■), 34 mM (○), and 60 mM (▲). (B) Replot of k_{obs} values from above. (C) Protection against inactivation by **1**. Inactivation at a single concentration of **1** (11.3 mM) in the presence of the indicated concentrations of 1-deoxynojirimycin: 0 μM (●); 63 μM (□).

Surprisingly, no time-dependent inactivation of human pancreatic α-amylase[16,43] was observed upon incubation with either of the 2,2-dihalomaltosyl chlorides **2** or **14**. This was initially surprising since the trinitrophenyl-2-deoxy-2,2-difluoromaltoside **18** had previously proven to be a slow, but effective time-dependent inactivator of the human amylase,[20] and since inherently, chloride should be a better leaving group than trinitrophenolate. Presumably binding interactions with the aryl moiety in the enzyme active site[44−45] accelerate, relatively, the reaction of amylase with the trinitrophenyl glycoside. Indeed, a similar phenomenon is seen with yeast α-glucosidase upon comparison of the inactivation parameters for the different inactivators. (Table 1) The trinitrophenyl-2,2-difluoroglucoside **16** ($k_i/K_i = 0.25\ \text{min}^{-1}\ \text{mM}^{-1}$) inactivates yeast α-glucosidase 2000-fold faster than does the corresponding α-chloride **10**. On that basis the lack of inactivation of human pancreatic α-amylase by the 2,2-difluoromaltosyl chlorides is not so surprising given that even the trinitrophenyl 2,2-difluoromaltoside is a slow ($k_i/K_i = 7.3 \times 10^{-3}\ \text{min}^{-1}\ \text{mM}^{-1}$) inactivator. Interestingly the replacement of a chlorine at the C-2 position by a fluorine in the 2,2-dihaloglucosyl chloride results in a 50-fold slower inactivation of yeast α-glucosidase. This rate difference is undoubtedly due, at least in part, to the greater electronegativity of fluorine than of chlorine thus a bigger inductive effect on the transition state, but may also be partly attributed to better

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TABLE 1. Summary of Inactivation Parameters for 2-Deoxy-2,2-dihaloglycosides with Two Model Enzymes

potential inactivator	enzyme	k_i/K_i (min^{-1} mM^{-1})
TNP22DFGlc (**16**)	yeast α-glucosidase	0.25[a]
2Cl2FGlcCl (**1**)	yeast α-glucosidase	$(5.3 \pm 3.4) \times 10^{-3}$ [b]
22DFGlcCl (**10**)	yeast α-glucosidase	$(9.1 \pm 3.3) \times 10^{-5}$ [b]
TNP22DFMal (**18**)	human pancreatic α-amylase	0.0073[a]
2Cl2FMalCl (**2**)	human pancreatic α-amylase	no inactivation[b]
22DFMalCl (**14**)	human pancreatic α-amylase	no inactivation[b]

[a] Reference 20. [b] This work.

complementarity of the equatorial 2-chloro substituent with the active site than is the case with an equatorial fluorine.

Conclusion

Convenient synthetic routes have been developed to a series of 2-deoxy-2,2-dihaloglycosyl derivatives of glucose and maltose involving common 2-fluoroglycal precursors. Useful mechanism-based inhibitors of α-glucosidases that function *via* the formation of relatively stable glycosyl−enzyme intermediates were thereby developed, though chloride leaving groups were not found to be very effective in trapping of intermediates, based on our kinetic experiments. Such reagents may be useful in the structural analysis of glycosyl−enzyme intermediates on α-glycosidases[46] and could also prove to have clinical importance as therapeutics that function by ablating α-glycosidase activities.

Experimental Section

3,4,6-Tri-*O*-acetyl-2-chloro-2-deoxy-2-fluoro-α-D-glucopyranosyl chloride (4). 3,4,6-tri-*O*-acetyl-2-fluoro-D-glucal (**3**) (0.80 g, 2.76 mmol)[31] was dissolved in dry carbon tetrachloride (150 mL) over 4 Å molecular sieves and cooled in a CCl_4/dry ice bath (−23 °C). Chlorine was bubbled through the solution until it turned a yellowish green (5 min). The flask was wrapped in aluminum foil to exclude light, allowed to slowly warm up to room temperature and stirred for 18 h. Upon completion of the reaction, excess chlorine was purged with a stream of dry nitrogen for several minutes until the solution was colorless, and the solvent was evaporated *in vacuo*. The resulting material was chromatographed twice (petroleum ether:diethyl ether = 2:1; then petroleum ether:diethyl ether = 3:1) giving the dihaloglycopyranosyl chloride **(4)** as a yellow oil (0.17 g, 0.47 mmol, 17%). ^{1}H NMR data ($CDCl_3$, 400 MHz): δ 6.06 (d, 1 H, J = 6.0 Hz), 5.71 (dd, 1 H, J = 22.8 Hz, J = 9.7 Hz), 5.28 (dt, 1 H, J = 9.7 Hz, J = 1.5 Hz), 4.4−4.05 (m, 3 H), 2.14 (s, 3 H), 2.07 (s, 3 H), 2.03 (s, 3 H). ^{19}F NMR data ($CDCl_3$, 188 MHz): δ −120.4 (dd, J = 22.8 Hz, J = 6.0 Hz). Anal. Calcd for $C_{12}H_{15}O_7FCl_2$: C, 39.91; H, 4.19; Found: C, 39.73; H, 4.08.

2-Chloro-2-deoxy-2-fluoro-α-D-glucopyranosyl Chloride (1). The acetylated glycopyranosyl chloride **(4)** (0.12 g, 0.33 mmol) was dissolved in HPLC grade methanol (10 mL) and cooled to 0 °C. Dry ammonia was bubbled in for 10 min. Then the ice bath was removed and the reaction was allowed to warm up to room temperature and further reacted for 4.5 h. Column chromatography (ethyl acetate:methanol:water = 27:2:1) gave a yellow solid which was further purified using ethyl acetate:methanol = 24:1 affording a yellow oil (0.039 g, 0.17 mmol, 50%). ^{1}H NMR data (D_2O, 200 MHz): δ 6.36 (d, 1 H, J = 6.6 Hz), 4.21 (dd, 1 H, J = 24.0 Hz, J = 9.0 Hz), 4.10−3.69 (m, 4 H). ^{19}F NMR data (D_2O, 188 MHz): δ −123.25 (dd, J = 24.0 Hz, J = 6.6 Hz). Anal. Calcd for $C_6H_9O_4FCl_2$: C, 30.66; H, 3.86; Found: C, 30.91; H, 3.93.

3,6-Di-*O*-acetyl-4-*O*-[2′,3′,4′,6′-tetra-*O*-acetyl-α-(1,4)-D-glucopyranosyl]-2-chloro-2-deoxy-2-fluoro-α-D-glucopyranosyl chloride (6). Protected 2-fluoro maltal (**5**) (0.80 g, 1.38 mmol) was dissolved in dry carbon tetrachloride (80 mL) over 4 Å molecular sieves and cooled in a CCl_4/dry ice bath (−23 °C). Chlorine was bubbled through the solution until it turned yellowish green (5 min), then the flask was wrapped in aluminum foil to exclude light, allowed to slowly warm up to room temperature and stirred overnight. Upon completion of the reaction, excess chlorine was purged with a stream of dry nitrogen for several minutes until the solution was colorless, and the solvent was evaporated *in vacuo*. The resulting material was chromatographed twice (petroleum ether: ethyl acetate = 3:2; then petroleum ether:diethyl ether = 2:3), giving the disaccharide chloride as a white foam (0.44 g, 0.68 mmol, 49%) ^{1}H NMR data ($CDCl_3$, 300 MHz): δ 6.03 (d, 1 H, J = 6.1 Hz), 5.83 (dd, 1 H, J = 23.3 Hz, J = 9.2 Hz), 5.37 (d, 1 H, J = 4.0 Hz), 5.36 (t, 1 H, J = 10.6 Hz), 5.08 (t, 1 H, J = 9.7 Hz), 4.88 (dd, 1 H, J = 10.6 Hz, J = 4.0 Hz), 4.59 (dd, 1 H, J = 12.5 Hz, J = 2.4 Hz) 4.50−4.20 (m, 4 H), 4.06 (dd, 1 H, J = 13.4 Hz, J = 2.3 Hz), 3.98 (dt, 1 H, J = 9.7 Hz, J = 2.3 Hz), 2.18 (s, 3 H), 2.15 (s, 3 H), 2.10 (s, 3 H), 2.09 (s, 3 H), 2.03 (s, 3 H), 2.01 (s, 3 H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 170.8, 170.6, 170.4, 170.0, 169.6, 169.5, 108.6 (d, J = 248.0 Hz), 96.1, 91.5 (d, J = 30.7 Hz), 73.1 (d, J = 17.9 Hz), 71.7, 71.4, 70.1, 69.3, 68.9, 68.0, 61.8, 61.5, 20.9, 20.8 (3C), 20.7 (2C). ^{19}F NMR data ($CDCl_3$, 282 MHz): δ −119.9 (dd, J = 23.0 Hz, J = 5.0 Hz) HRMS data: calcd for $C_{24}H_{31}O_{15}F^{35}Cl_2 + Na^+$, 671.0922; found, 671.0923.

2-Chloro-2-deoxy-2-fluoro-4-*O*-[α-(1,4)-D-glucopyranosyl]-α-D-glucopyranosyl chloride (2). Protected disaccharide chloride (**6**) (80 mg, 0.12 mmol) was dissolved in 10 mL of dry methanol, a small piece of sodium metal was added then the mixture was stirred at room temperature for 2 h. Acidic ion-exchange resin was added, stirred for 10 min until the solution became weakly acidic, then the resin was filtered off and the solvent was evaporated *in vacuo*. The product was purified by flash column chromatography (ethyl acetate:methanol:water = 7:2:1) yielding **(2)** (41 mg, 0.10 mmol, 82%) as a white foam. ^{1}H NMR data (D_2O, 300 MHz): δ 6.26 (d, 1 H, J = 6.3 Hz), 5.35 (d, 1 H, J = 4.0 Hz), 4.40 (dd, 1 H, J = 24.3 Hz, J = 9.8 Hz), 4.13 (dt, 1 H, J = 9.8 Hz, J = 2.3 Hz), 3.90 (t, 1 H, J = 9.8 Hz), 3.77−3.53 (m, 6 H), 3.45 (dd, 1 H, J = 10.0 Hz, J = 4.0 Hz), 3.32 (t, 1 H, J = 9.7 Hz). ^{13}C NMR data (D_2O, 100 MHz): δ 110.52 (d, J = 242.0 Hz), 99.71, 91.79 (d, J = 31.0 Hz), 73.86, 73.83, 73.68, 72.99, 72.86, 71.64, 69.40, 60.53, 59.98. ^{19}F NMR data (D_2O, 282 MHz): δ −123.87 (dd, J = 24.3 Hz, J = 6.3 Hz) HRMS data: calcd for $C_{12}H_{19}O_9FCl_2 + Na^+$, 419.0288; found, 419.0289.

1,3,4,6-Tetra-*O*-acetyl-2-deoxy-2,2-difluoro-α-D-arabinohexopyranose (7) (Acetyl Hypofluorite Method). Sodium acetate (280 mg), glacial acetic acid (3.5 mL) and $CFCl_3$ (35 mL) were mixed in a 3-necked round-bottom flask and cooled in a dry ice/acetone bath (−78 °C). A gas reservoir (~1 L) was filled with 20% fluorine in neon (10 psi) which was diluted 4-fold with helium to 40 psi. This mixture was then bubbled through the slurry. The flask was thus charged twice before compound (**3**) (0.800 g, 2.76 mmol) dissolved in $CFCl_3$ (10 mL) was added to the mechanically stirred mixture. The loosely stoppered flask was allowed to warm to room temperature. After general workup, column chromatography (pe-

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troleum ether: ethyl acetate = 4:1) afforded the product **7** (0.77 g, 2.1 mmol, 76%) as an anomeric mixture, only the spectrum of the α-anomer is given here. ^{1}H NMR data ($CDCl_3$, 200 MHz): δ 6.19 (t, 1 H, J = 3.0 Hz), 5.50 (dt, 1 H, J = 20.0 Hz, J = 10.0 Hz), 5.20 (t, 1 H, J = 10.0 Hz), 4.3−4.0 (m, 3 H), 3.88 (s, 3 H), 3.85 (s, 3 H), 3.81 (s, 3 H), 3.74 (s, 3 H). ^{19}F NMR data ($CDCl_3$, 188 MHz): δ −121.0 (F-2/eq., F-2/ax. co-incident). MS: calcd for $C_{14}H_{18}F_2O_9 + Na^+$, 391.1; found, 391.0.

3,4,6-Tri-*O*-acetyl-2-deoxy-2,2-difluoro-α-D-arabinohexopyranose (8). Fully acetylated difluorosugar (**7**) (0.5 g, 1.36 mmol) was dissolved in DMF (10 mL), hydrazine acetate (280 mg, 3.0 mmol) was added and allowed to react 3 days at 50 °C. Evaporation of the solvent *in vacuo*, followed by flash chromatography (hexanes: ethyl acetate = 1:1) afforded the hemiacetal (280 mg, 66%) as a colorless gum. ^{1}H NMR data ($CDCl_3$, 300 MHz): δ 5.56 (ddd, 1 H, J = 19.0 Hz, J = 10.0 Hz, J = 6.0 Hz), 5.24−5.10 (m, 2 H), 4.32−4.00 (m, 3 H), 3.48 (broad, 1 H), 2.09 (s, 3 H), 2.06 (s, 3 H), 2.00 (s, 3 H). ^{13}C NMR data ($CDCl_3$, 75 MHz): δ 171.1, 170.0, 169.5, 115.6 (dd, J = 257.0 Hz, J = 246.0 Hz), 91.4 (dd, J = 36.0 Hz, J = 28.7 Hz), 68.7 (t, J = 18.5 Hz), 68.2, 67.7 (d, J = 6.3 Hz), 61.9, 20.8, 20.7, 20.6 ^{19}F NMR data ($CDCl_3$, 188 MHz): δ −120.7 (dd, J = 251.0 Hz, J = 6.0 Hz), −122.7 (ddd, J = 251.0 Hz, J =19 Hz, J = 5.0 Hz). HRMS: calcd for $C_{12}H_{16}F_2O_8 + Na^+$, 349.0711; found, 349.0710.

3,4,6-Tri-*O*-acetyl-2-deoxy-2,2-difluoro-α-D-arabinohexopyranose (8) (Selectfluor Method). Protected 2-fluoroglucal **3** (0.64 g, 2.2 mmol) was dissolved in 25 mL of CH_3NO_2/water (v/v = 4:1) mixed solvent, and Selectfluor (1.17 g, 3.3 mmol) was added. The reaction mixture was stirred at room temperature for overnight and then was heated to 95 °C for 4 h. After the mixture had cooled down, most of the solvent was evaporated under diminished pressure. The residue was redissolved in EtOAc and washed successively with water, saturated $NaHCO_3$(aq), and brine. After drying over $MgSO_4$ and evaporation of the solvent, flash chromatography (petroleum ether: ethyl acetate = 1:1) yielded a colorless syrup of **8** (130 mg, 20%).

3,4,6-Tri-*O*-acetyl-2-deoxy-2,2-difluoro-α-D-arabinohexopyranosyl chloride (9). The hemiacetal (**8**) (0.079 g, 0.23 mmol) was dissolved in freshly distilled thionyl chloride (1 mL, 11 mmol) and stirred at refluxing (∼65 °C) for 80 h. Column chromatography (petroleum ether:ethyl acetate = 5:1) yielded the acetylated glycosyl chloride (0.049 g, 0.14 mmol, 62%). ^{1}H NMR data ($CDCl_3$, 300 MHz): δ 5.92 (dd, 1 H, J = 4.8 Hz, J = 2.0 Hz), 5.68 (dt, 1 H, J = 15.0 Hz, J = 10.0 Hz), 5.19 (t, 1 H, J = 10.0 Hz), 4.2−4.0 (m, 3 H), 2.13 (s, 3 H), 2.09 (s, 3 H), 2.05 (s, 3 H). ^{13}C NMR data ($CDCl_3$, 75 MHz): δ 170.6, 169.6, 169.2, 115.3 (dd, J = 257.4 Hz, J = 252.6 Hz), 88.2 (t, J = 34.0 Hz), 71.1, 68.1 (t, J = 19.4 Hz), 66.7 (d, J = 4.2 Hz), 61.0, 20.7, 20.6, 20.5. ^{19}F NMR data ($CDCl_3$, 188 MHz): δ −115.5 (ddd, J = 249.8 Hz, J = 15.0 Hz, J = 4.8 Hz), −116.9 (ddd, J = 249.8 Hz, J = 10.0 Hz). Anal. Calcd for $C_{12}H_{15}O_7F_2Cl$: C, 41.81; H, 4.39. Found: C, 41.76; H, 4.50.

2-Deoxy-2,2-difluoro-α-D-arabinohexopyranosyl Chloride (10). The acetylated glycosyl chloride **(9)** (0.049 g, 0.14 mmol) was dissolved in methanol (10 mL) and cooled to 0 °C, and dry ammonia gas was bubbled in for 5 min. The reaction was then left stirring at room temperature for overnight. The resulting light orange oil was purified by column chromatography (ethyl acetate: methanol: water = 147: 2: 1) to afford compound **10** as a colorless oil (0.016 g, 0.073 mmol, 52%). ^{1}H NMR data (D_2O, 300 MHz): δ 6.15 (d, 1 H, J = 7.6 Hz), 4.25 (ddd, 1 H, J = 21.0 Hz, J = 9.5 Hz, J = 5.0 Hz), 3.92−3.62 (m, 4 H). ^{13}C NMR data ($CDCl_3$, 75 MHz): δ 170.6, 169.6, 169.2, 115.3 (dd, J = 258.0 Hz, J = 253.0 Hz), 88.2 (t, J = 34.0 Hz), 71.1, 68.1 (t, J = 19.4 Hz), 66.7 (d, J = 4.2 Hz), 61.0, 20.7, 20.6, 20.5. ^{19}F NMR data (D_2O, 188 MHz): δ −116.1 (dd, J = 248.7 Hz, J = 5.0 Hz), −119.45 (ddd, J = 248.7 Hz, J = 21.0 Hz, J = 6.0, Hz). Anal. Calcd for $C_6H_9O_4F_2Cl$: C, 32.97; H, 4.15; Found: C, 33.15; H, 4.16.

1,3,6-Tri-*O*-acetyl-4-*O*-[2′,3′,4′,6′-tetra-*O*-acetyl-α-(1,4)-D-glucopyranosyl]-2-deoxy-2,2-difluoro-α/β-D-arabinohexopyranose (11) (Acetyl Hypofluorite Method). Per-*O*-acetyl-2-fluoromaltal **5 (**0.99 g, 1.7 mmol) was fluorinated according to the general fluorination procedure mentioned in the Supporting Information. The reaction mixture was partially purified by flash chromatography (hexanes: ethyl acetate = 1: 1) to afford 0.67 g (1.0 mmol, 59%) of a syrup containing both α- and β anomers of **11**. Only the spectra of the α-anomer are given here. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 6.14 (d, 1 H, J = 4.1 Hz), 5.57 (dt, 1 H, J = 16.7 Hz, J = 8.4 Hz), 5.43 (d, 1 H, J = 3.9 Hz), 5.36 (t, 1 H, J = 9.8 Hz), 5.07 (t, 1 H, J = 9.8 Hz), 4.86 (dd, 1 H, J = 10.5 Hz, J = 3.9 Hz), 4.50−3.90 (m, 7 H), 2.24 (s, 3 H), 2.14 (s, 3 H), 2.13 (s, 3 H), 2.09 (s, 3 H), 2.06 (s, 3 H), 2.02 (s, 3 H), 2.00 (s, 3 H). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 170.8, 170.7, 170.6, 170.2, 169.8, 169.6, 168.0, 114.8 (dd, J = 258 Hz, J = 246 Hz), 96.0, 88.8 (dd, J = 37.7 Hz, J = 31.7 Hz), 71.5 (d, J = 4.5 Hz), 71.1 (t, J = 19.6 Hz), 70.7, 70.3, 69.3, 68.9, 68.0, 62.2, 61.5, 21.0, 20.94, 20.91, 20.88, 20.80 (3C). HRMS: calcd for $C_{26}H_{34}F_2O_{17} + Na^+$, 679.1662; found, 679.1650. This anomeric mixture was directly used in the next step without further purification.

3,6-Di-*O*-acetyl-4-*O*-[2′,3′,4′,6′-tetra-*O*-acetyl-α-(1,4)-D-glucopyranosyl]-2-deoxy-2,2-difluoro-α-D-arabinohexopyranose (12). To 0.67 g (1.0 mmol) of syrup **11** was added 0.13 g (1.4 mmol, 1.4 equiv) of hydrazine acetate in DMF (20 mL), and the reaction was continued for 3 days at 50 °C. The reaction was stopped by redissolving the mixture in ethyl acetate then the organic layer was washed successively with water and brine. Evaporation of the solvent *in vacuo*, followed by flash chromatography (hexanes:ethyl acetate = 1:1) gave the α-hemiacetal **12** (0.42 g, 0.68 mmol, 68%) as a white foam. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 5.63 (ddd, 1 H, J = 18.5 Hz, J = 9.2 Hz, J = 6.5 Hz), 5.42 (d, 1 H, J = 4.0 Hz), 5.35 (dd, 1 H, J = 10.4 Hz, J = 9.6 Hz), 5.18 (d, 1 H, J = 4.7 Hz), 5.05 (t, 1 H, J = 9.8 Hz), 4.84 (dd, 1 H, J = 10.4 Hz, J = 4.0 Hz), 4.6−4.0 (m, 7 H), and 2.2−1.9 (5 s, 6 OAc). ^{13}C NMR data ($CDCl_3$, 75 MHz): δ 170.9, 170.8 (2C), 170.2, 169.8, 169.6, 115.8 (dd, J = 258.0 Hz, J = 245.0 Hz), 95.7, 91.0 (dd, J = 35.0 Hz, J = 28.8 Hz), 71.9 (d, J = 5.4 Hz), 70.9 (t, J = 19.0 Hz), 70.2, 69.5, 68.6, 68.5, 68.1, 62.6, 61.5, 20.93, 20.85, 20.79, 20.7 (3C) ^{19}F NMR ($CDCl_3$, 282 MHz): δ −120.9 (dd, J = 253.0 Hz, J = 6.3 Hz), −122.7 (ddd, J = 253.0 Hz, J = 18.4 Hz, J = 4.5 Hz). HRMS data: calcd for $C_{24}H_{32}F_2O_{16} + Na^+$, 637.1556; found, 637.1562.

3,6-Di-*O*-acetyl-4-*O*-[2′,3′,4′,6′-tetra-*O*-acetyl-α-(1,4)-D-glucopyranosyl]-2-deoxy-2,2-difluoro-α-D-arabinohexopyranose (12) (Selectfluor Method). Protected 2-fluoromaltal **5** (20 mg, 0.035 mmol) and 18 mg (0.05 mmol) of Selectfluor were dissolved in 5 mL of CH_3NO_2/water (v/v = 4:1) mixed solvent. The reaction mixture was stirred at room temperature overnight and then heated to 95 °C for 4 h. After cooling down the mixture, most of the solvent was evaporated under diminished pressure. The residue was redissolved in EtOAc and washed successively with water, saturated $NaHCO_3$(aq) and brine. After drying over $MgSO_4$ and evaporation of the solvent, flash chromatography (petroleum ether: ethyl acetate = 1: 1) yielded colorless syrup **12** (18 mg, 84%).

3,6-Di-*O*-acetyl-4-*O*-[2′,3′,4′,6′-tetra-*O*-acetyl-α-(1,4)-D-glucopyranosyl]-2-deoxy-2,2-difluoro-α-D-arabinohexopyranosyl Chloride (13). The hemiacetal **12** (250 mg, 0.41 mmol) was dissolved in 10 mL of dry CH_2Cl_2 along with 3.5 mL of $SOCl_2$ (large excess to prevent the evaporation of $SOCl_2$) and then BiOCl (0.35 g) was added. The reaction flask was wrapped with aluminum foil to exclude light, and then the mixture was stirred vigorously at room temperature under a nitrogen atmosphere for 3 days when TLC showed that most of the starting material had been consumed. The reaction mixture was poured into ice-cold water, stirred, then transferred to a separatory funnel. The organic phase was washed successively with water, saturated $NaHCO_3$(aq), water, and brine. The organic layer was dried with $MgSO_4$, filtered and the solvent was evaporated *in vacuo*. Two rounds of flash column chromatography (hexanes: ethyl acetate = 1:1 then $CHCl_3$: acetone = 20:1) yielded the pure product (34 mg, 0.054 mmol, 13%) as a colorless syrup. ^{1}H NMR data ($CDCl_3$, 300 MHz): δ 5.93 (d, 1 H,

J = 6.4 Hz), 5.78 (ddd, 1 H, J = 18.8 Hz, J = 9.6 Hz, J = 5.2 Hz), 5.45 (d, 1 H, J = 4.0 Hz), 5.37 (t, 1 H, J = 10.4 Hz), 5.09 (t, 1 H, J = 10.0 Hz), 4.87 (dd, 1 H, J = 10.4 Hz, J = 4.0 Hz), 4.59 (dd, 1 H, J = 12.6 Hz, J = 2.4 Hz), 4.34 (dt, 1 H, J = 9.6 Hz, J = 2.4 Hz), 4.29−4.23 (m, 2 H), 4.18 (t, 1H, J = 9.6 Hz), 4.08 (dd, 1 H, J = 12.6 Hz, J = 2.4 Hz), 3.95 (dt, 1 H, J = 10.0 Hz, J = 2.4 Hz), 2.17 (s, 3 H), 2.16 (s, 3 H), 2.11 (s, 3 H), 2.08 (s, 3 H), 2.04 (s, 3 H), 2.02 (s, 3 H). ^{13}C NMR data ($CDCl_3$, 100 MHz): δ 171.6, 171.5, 171.2, 170.8, 170.4, 170.3, 116.2 (dd, J = 258.6 Hz, J = 249.5 Hz), 96.9, 88.8 (t, J = 35.0 Hz), 72.3, 72.0 (d, J = 5.4 Hz), 71.1, 71.0 (t, J = 19.3 Hz), 70.2, 69.8, 68.9, 62.6, 62.3, 21.7, 21.63, 21.61, 21.56 (3C). ^{19}F NMR data ($CDCl_3$, 282 MHz): δ −115.8 (ddd, J = 247.5 Hz J = 18.8 Hz, J = 7.0 Hz) 116.9 (dd, J = 247.5 Hz, J = 5.2 Hz) HRMS data: calcd for $C_{24}H_{31}O_{15}{}^{35}ClF_2 + Na^+$, 655.1217; found, 655.1215.

2-Deoxy-2,2-difluoro-4-*O*-[α-(1,4)-D-glucopyranosyl]-α-D-arabinohexopyranosyl Chloride (14). Protected disaccharide **13** (20 mg, 0.03 mmol) was dissolved in 5 mL of methanol (HPLC grade), cooled to 0 °C and dry ammonia was bubbled in for 1 min. After removal of the ice bath the reaction was stirred at room temperature overnight. The solvent was then evaporated to dryness and the residue purified by flash column chromatography (ethyl acetate: methanol:water = 7:2:1) to afford **14** (10 mg 0.026 mmol, 83%) as a white foam. ^{1}H NMR data (D_2O, 400 MHz): δ 6.13 (d, 1 H, J = 7.3 Hz), 5.36 (d, 1 H, J = 3.8 Hz), 4.46 (ddd, 1 H, J = 21.2 Hz, J = 9.2 Hz, J = 5.5 Hz), 4.07 (dt, 1 H, J = 9.2 Hz, J = 3.0 Hz), 3.85 (t, 1 H, J = 9.2 Hz), 3.77−3.52 (m, 6 H), 3.44 (dd, 1 H, J = 10.0 Hz, J = 3.8 Hz), 3.31 (t, 1 H, J = 9.1 Hz). ^{13}C NMR data (D_2O, 100 MHz): δ 119.42 (t, J = 244.0 Hz), 102.24, 91.05 (t, J = 36.0 Hz), 76.32, 76.15, 75.71, 75.59, 74.39, 73.14 (t, J = 19.0 Hz), 72.14, 63.28, 62.65. ^{19}F NMR data (D_2O, 282 MHz): δ −117.7 (dd, J = 249.0 Hz, J = 5.5 Hz) 119.7 (ddd, J = 249.0 Hz, J = 21.2 Hz, J = 7.3 Hz). HRMS data: calcd for $C_{12}H_{19}{}^{35}ClF_2O_9 + Na^+$, 403.0583; found, 403.0589.

2,4,6-Trinitrophenyl-3,4,6-tri-*O*-acetyl-2-deoxy-2,2-difluoro-α-D-arabinohexopyranoside (15). To a solution of the difluorohemiacetal **8** (93 mg, 0.28 mmol) dissolved in dry CH_2Cl_2 (1 mL) were added 2,6-di-*tert*-butyl pyridine (0.1 mL, 0.44 mmol) and fluoro-2,4,6-trinitrobenzene (90 mg, 0.39 mmol), and the mixture was allowed to stir for 10 days at room temperature in the dark under N_2. Evaporation of the solvent *in vacuo*, followed by flash chromatography (hexanes:ethyl acetate = 1:2) afforded 2,4,6-trinitrophenyl 3,4,6-tri-*O*-acetyl-2-deoxy-2,2-difluoro-α-D-arabinohexopyranoside **15** (76 mg, 51%) as a yellowish gum. ^{1}H NMR ($CDCl_3$): δ 8.84 (s, 2 H), 5.68 (ddd, 1 H, J = 19.2 Hz, J = 9.9 Hz, J = 6.0 Hz), 5.62 (d, 1 H, J = 5.0 Hz), 5.23 (dt, 1 H, J = 10.0 Hz, J = 1.4 Hz), 4.3−4.0 (m, 3 H), 2.2−2.0 (2 s, 3 OAc). ^{19}F NMR ($CDCl_3$): δ −118.2 (ddd, J = 263 Hz, J = 19.0 Hz, J = 6.0 Hz), −121.3 (dd, J = 263.0 Hz, J = 6.0 Hz).

2,4,6-Trinitrophenyl-2-deoxy-2,2-difluoro-α-D-arabinohexopyranoside (16) To the protected difluoroglycoside **15** (84 mg, 0.18 mmol) in dry MeOH (2 mL) at 0 °C was added freshly distilled acetyl chloride (80 μL), and the mixture was allowed to stir at 4 °C for 2 days. After workup, flash chromatography (hexanes: ethyl acetate: = 1:1) afforded pure 2,4,6-trinitrophenyl-2-deoxy-2,2-difluoro-α-D-arabinohexopyranoside (14 mg, 19%) as a yellowish gum, which was freeze-dried to give a yellowish white solid. Its characterization is consistent with an earlier description.[20]

2,4,6-Trinitrophenyl-3,6-di-*O*-acetyl-4-*O*-[2′,3′,4′,6′-tetra-*O*-acetyl-α-(1,4)-D-glucopyranosyl]-2-deoxy-2,2-difluoro-α-D-arabinohexopyranoside (17). To a solution of protected difluorohemiacetal **12** (535 mg, 0.87 mmol) dissolved in dry CH_2Cl_2 (50 mL) were added 2,6-di-*tert*-butyl pyridine (0.6 mL, 2.7 mmol) and fluoro-2,4,6-trinitrobenzene (460 mg, 2.0 mmol), and the mixture was allowed to stir for 10 days at room temperature in the dark under N_2. Evaporation of the solvent *in vacuo*, followed by flash chromatography (hexanes: ethyl acetate = 1: 1) afforded 2,4,6-trinitrophenyl-3,6-di-*O*-acetyl-4-*O*-[2′,3′,4′,6′-tetra-*O*-acetyl-α-(1,4)-D-glucopyranosyl]-2-deoxy-2,2-difluoro-α-D-arabinohexopyranoside (668 mg, 91%) as a yellowish gum. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.85 (s, 2 H), 5.71 (ddd, 1 H, J = 20.3 Hz, J = 8.8 Hz, J = 3.6 Hz), 5.61 (d, 1 H, J = 5.3 Hz), 5.44 (d, 1 H, J = 4.0 Hz), 5.33 (t, 1 H, J = 10.0 Hz), 5.02 (t, 1 H, J = 9.9 Hz), 4.83 (dd, 1 H, J = 10.5 Hz, J = 4.0 Hz), 4.4−3.9 (m, 7 H), and 2.2−1.9 (6 s). ^{13}C NMR data ($CDCl_3$, 100 MHz): δ 171.7, 171.5, 171.1, 170.9, 170.4, 170.0, 145.75, 145.73, 143.78, 124.7 (3 × C), 114.9 (dd, J = 262.0 Hz, J = 246.1 Hz), 100.9 (dd, J = 40.2 Hz, J = 28.9 Hz), 96.7, 73.5, 74.3 (d, J = 5.6 Hz), 71.0, 70.8 (t, J = 20.3 Hz), 70.2, 69.8, 68.9, 62.8, 62.4, 21.64 (2 × CH$_3$CO), 21.59, 21.55 (3 × CH$_3$-CO). ^{19}F NMR ($CDCl_3$): δ −126.0 (ddd, J = 264.0 Hz, J = 19.3 Hz, J = 5.1 Hz), −128.5 (dd, J = 264.0 Hz, J = 4.7 Hz). HRMS data: calcd for $C_{30}H_{33}F_2N_3O_{22} + Na^+$, 848.1421; found, 848.1416.

2,4,6-Trinitrophenyl-2-deoxy-2,2-difluoro-4-*O*-[α-(1,4)-D-glucopyranosyl]-α-D-arabinohexopyranoside (18). To acetylated difluoroglycoside **17** (96 mg, 0.12 mmol) in dry MeOH (2 mL) at 0 °C was added freshly distilled acetyl chloride (80 μL), and the mixture was allowed to stir at 4 °C for 2 days. After workup, flash chromatography (ethyl acetate: methanol: water = 20: 2: 1) afforded **18** (47 mg, 71%) as a yellowish gum, which was freeze-dried to give a yellowish white solid. Its characterization is consistent with an earlier description.[20]

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Supporting Information Available: Text giving general experimental methods, including the synthesis of precursor **5** and enzyme kinetic studies and figures showing ^{1}H and ^{13}C NMR spectra of compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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