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Syntheses of 2-deoxy-2-fluoro mono- and oligo-saccharide glycosides from glycals and evaluation as glycosidase inhibitors

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ABSTRACT

Several fluorinated oligosaccharides, including 2-deoxy-2-fluoro derivatives of cellobiose, maltose, and maltotriose were synthesized by the action of fluorine or acetyl hypofluorite on the corresponding glycal peracetates. Temperature effects on the stereoselectivities of these reactions were examined. Addition of acetyl hypofluorite to several 2-substituted glycals in the *gluco* or *galacto* series gave 2,2-disubstituted *arabino*- or *lyxo*-hexose derivatives; 3,4,6-tri-*O*-acetyl-2-fluoro-D-glucal or the analogous galactal yielded 2-deoxy-2,2-difluoro *arabino*- or *lyxo*-hexose peracetates, whereas 2-acetoxy-3,4,6-tri-*O*-acetyl-D-glucal or the analogous galactal gave 2(*R*)-2-acetoxy-2-fluoro-*arabino*- or *lyxo*-hexose peracetates, respectively. 2-Acetamido-3,4,6-tri-*O*-acetyl-D-glucal gave 2(*R*)-2-acetamido-2-acetoxy-3,4,6-tri-*O*-acetyl-α-D-*arabino*-hexopyranosyl fluoride. 2,4-Dinitrophenyl 2-deoxy-2-fluoro-β-cellobioside was an inactivator of the exoglucanase from *Cellulomonas fimi* while 2-deoxy-2-fluoro-α-maltosyl and α-maltotriosyl fluorides were slow substrates of human pancreatic alpha-amylase and rabbit muscle glycogen debranching enzyme, respectively.

INTRODUCTION

The addition of acetyl hypofluorite (AcOF) or elemental fluorine to simple pyranoid or furanoid glycals to produce 2-deoxy-2-fluoro derivatives has been extensively studied[1–4]. Acetyl hypofluorite has subsequently seen widespread application in the synthesis of 2-deoxy-2-[^{18}F]fluoro-D-glucose (FDG) for positron emission tomographic (PET) imaging[5,6]. However, the extension of such electrophilic fluorination methodology to oligosaccharide glycals to give the corresponding 2-deoxy-2-fluorooligosaccharides has not been described. Reports of fluorinations of 2-substituted glycals are also uncommon, although the addition of

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trifluoromethyl hypofluorite (CF_3OF) to 2,3,6-trideoxy-2-fluoro-3-(trifluoroacetamido)-L-galactal derivatives[7], to 4-*O*-benzyl-6-deoxy-2-fluoro-3-*O*-methyl-L-glucal[8], or to 3,4,6-tri-*O*-acetyl-2-cyano-D-galactal[9] has been reported to give the corresponding 2-deoxy-2,2-difluoro-L- or 2-cyano-2-fluoro-D-derivatives as mixtures of trifluoromethyl glycosides and glycosyl fluorides. Indeed, the earliest synthesis of 2-deoxy-2,2-difluoro-D-*arabino*-hexose ("2,2-difluoro-D-glucose") described the addition of trifluoromethyl hypofluorite to 3,4,6-tri-*O*-acetyl-2-deoxy-2-fluoro-D-glucal to give an anomeric mixture of 2,2-difluoro *arabino*-hexosyl fluorides and the trifluoromethyl β-glycoside[10]. Such compounds in which a fluorine has been substituted at the 2-position of a glycopyranose ring are of interest as precursors of potential glycosidase inhibitors and probes of the enzymic mechanism.

"Retaining" glycosidases catalyze the hydrolysis of glycosidic bonds with overall retention of anomeric configuration. The mechanism involves the formation and hydrolysis of a glycosyl–enzyme intermediate via transition states with substantial oxocarbonium-ion character. 2-Deoxy-2-fluoroglycosides having good leaving groups have been shown to act as covalent, mechanism-based inhibitors of retaining β-glycosidases by forming a relatively stable 2-deoxy-2-fluoro glycosyl–enzyme intermediate[11,12]. The fluorine at C-2 of the inhibitor is proposed to inductively destabilize the positively charged transition states, slowing the rates of both glycosyl–enzyme formation and hydrolysis, while the good leaving group increases only the rate of glycosyl–enzyme formation, thereby resulting in accumulation of the intermediate and inactivation of the enzyme.

In efforts to more fully understand this inactivation process, we have shown in recent studies of *Escherichia coli* (*lacZ*) β-galactosidase[13] and *Agrobacterium faecalis* β-glucosidase[14], using series of specifically deoxygenated and fluorinated glycopyranosides that it is only through substitution of the 2-hydroxyl group, preferably with small, electronegative elements such as fluorine, that the glycosyl–enzyme intermediate is sufficiently stabilized (at least with these glycosidases) to result in inactivation of the enzyme. We therefore decided to investigate the syntheses of several 2,2-disubstituted glycosides (in which at least one of the substituents was a fluorine atom) as potential glycosidase inhibitors by fluorination of the corresponding 2-substituted glycals with acetyl hypofluorite.

Extension of this approach to enzymes which degrade polysaccharides would be of considerable interest. Such studies require the use of 2-deoxy-2-fluorooligosaccharides with good leaving groups. This paper describes the syntheses of several 2-deoxy-2-fluorooligosaccharides by the same approach, as well as preliminary kinetic studies on the enzymic modes of action.

RESULTS AND DISCUSSION

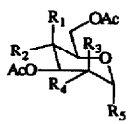
The starting 2-substituted monosaccharide glycals, 2-acetamido-3,4,6-tri-*O*-acetyl-D-glucal[15], 2-acetoxy-3,4,6-tri-*O*-acetyl-D-glucal[16], and the analogous galactal, were synthesized according to published procedures. 3,4,6-Tri-*O*-acetyl-2-flu-

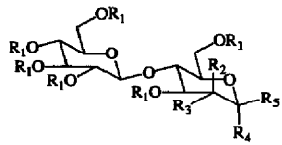
oro-D-glucal and the analogous galactal were synthesized from trifluoromethyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-fluoro α-glucoside or 3,4,6-tri-*O*-acetyl-2-deoxy-2-fluoro-α-D-galactosyl fluoride[17], respectively, by conversion into the α-glycosyl bromides with HBr–acetic acid, followed by elimination of HBr with triethylamine. 3,4,6-Tri-*O*-acetyl-2-chloro-D-glucal, identified by comparison of its ^{1}H NMR spectrum with literature data[18], was isolated as an elimination product in the reaction of 2-chloro-2-deoxy-α-D-glucopyranosyl bromide[19] with silver fluoride. Syntheses of the glycal derivatives of the β-(1 → 4)-linked disaccharide cellobiose[20], and the α-(1 → 4)-linked di- and tri-saccharides maltose and maltotriose were accomplished by published procedures[21].

Reaction of the 2-acetoxy monosaccharide glycals with acetyl hypofluorite resulted in the formation of 2(*R*)-2-acetoxy-2-fluoro products. In the monofluorinated compounds **1** and **2**, fluorine–proton coupling constants for H-3 ranged from 6–8 Hz, whereas those for H-1 were near zero, consistent with an equatorial fluorine atom at C-2 vicinal to an equatorial proton at C-1. Thus the only addition products isolated were the α anomers having the (*R*) configuration at C-2, suggesting that the addition of acetyl hypofluorite to these 2-substituted glycals, like that with the parent glycals, is highly stereoselective (e.g., ~ 19:1 *syn* addition to the α face of 3,4,6-tri-*O*-acetyl-D-glucal in $CFCl_3$)[3,4]. Yields of these and the other fluorinated products below were generally modest, presumably because of decomposition or formation of minor side-products associated with the use of highly reactive acetyl hypofluorite or elemental fluorine.

Similarly, reaction of per-*O*-acetylated 2-fluoro-D-galactal or 2-fluoro-D-glucal with acetyl hypofluorite gave 2-deoxy-2,2-difluoro products in the *lyxo* or *arabino* series. The *lyxo*-hexose **4** was treated with HBr in acetic acid to give the 2,2-difluoro α-D-*lyxo*-hexopyranosyl bromide **5**, and the *arabino*-hexose **7** reacted with 1-fluoro-2,4-dinitrobenzene (FDNB) to give the 2,2-difluoro α-D-*arabino*-hexopyranoside **8**. ^{1}H NMR spectra of the 2-deoxy-2,2-difluoro *lyxo*-hexoside **5** and the *arabino*-hexoside **8** each showed 3–7 Hz doublets for H-1, consistent with axial fluorine at C-2 vicinal to an equatorial proton at C-1, while H-3 showed the expected couplings to the axial and equatorial C-2 fluorines. The small value of $J_{3,Fa}$ for the *arabino*-hexoside is consistent with that reported for other 2-deoxy-2,2-difluoro *arabino*-hexosides[22]. The ^{1}H decoupled ^{19}F NMR spectra of **5** and **8** each showed two doublets, exhibiting strong second-order effects (particularly pronounced in the *arabino*-hexoside) attributable to the large geminal couplings between the two fluorines $J_{Fe,Fa}$ 257 and 260 Hz, respectively). Reaction of 3,4,6-tri-*O*-acetyl-2-chloro-D-glucal with acetyl hypofluorite under identical conditions to those used for the 2-fluoroglycals above gave a complex mixture of products from which none of the expected 2(*R*)- or 2(*S*)-2-chloro-2-fluoro compounds were isolated.

The major product from addition of acetyl hypofluorite to 2-acetamido-3,4,6-tri-*O*-acetyl-D-glucal was 2(*R*)-2-acetamido-2-acetoxy-3,4,6-tri-*O*-acetyl-α – D-*arabino*-hexopyranosyl fluoride **9**, resulting from fluorination at C-1 rather than C-2. In





	R_1	R_2	R_3	R_4	R_5		R_1	R_2	R_3	R_4	R_5
1	H	OAc	OAc	F	OAc	**10**	Ac	H	F	OAc	H
2	OAc	H	OAc	F	OAc	**11**	Ac	F	H	H	OAc
4	OAc	H	F	F	OAc	**12**	Ac	H	F	H/OH	H/OH
5	OAc	H	F	F	Br	**13**	Qc	H	F	H	ODNP
6	H	OAc	F	F	OAc	**14**	H	H	F	H	ODNP
7	H	OAc	F	F	OH						
8	H	OAc	F	F	ODNP						
9	H	OAc	NHAc	OAc	F						

contrast to the previously described addition products, **9** exhibited a 51-Hz geminal fluorine coupling to H-1, while a 1.8-Hz fluorine coupling was observed to H-3. The magnitude of this smaller coupling is consistent with a four-bond coupling between an axial fluorine and an axial proton in a 1,3-*cis* relationship as in methyl 3-deoxy-3-fluoro-β-D-allopyranoside ($J_{1,F}$ 2.0 Hz)[23]. If the anomeric fluorine of **9** was equatorial rather than axial, the structure would be analogous to a 3-deoxy-3-fluoro-β-D-glucopyranoside, in which the fluorine couples much less strongly to H-1 ($J_{1,F}$ 0–0.5 Hz)[24,25]. Assuming *syn* addition, the C-2 of **9** was therefore assigned the (*R*) configuration. This unusual result might be anticipated since the C-2 carbon in the *N*-acetamido derivative is more electropositive than in the parent glycal due to stabilization of the developing carbonium ion at C-2 by electron donation from the amide nitrogen, such stabilization evidently being greater than that afforded at C-1 by the endocyclic oxygen. Thus the addition of electrophilic fluorine occurs in the opposite sense with regard to the parent glycal, addition of fluorine occurring at C-1 rather than at C-2.

Treatment of 3,6,2′,3′,4′,6′-hexa-*O*-acetylcellobial[20], a β(1 → 4)-linked disaccharide derivative, with acetyl hypofluorite in 10:1 $CFCl_3$–acetonitrile at 0°C in the usual manner afforded a 1.5:1 mixture (by ^1H NMR) of the 2-deoxy-2-fluorocellobiose derivative **10** and the β-addition product **11**, both identified by their ^1H coupled–^{19}F NMR spectra. Cooling the mixture to −78°C increased the ratio of α/β addition to 5:1. Since treatment of tri-*O*-acetyl-D-glucal under similar conditions yields a much greater ratio of α/β addition (19:1 in $CFCl_3$ at room temperature)[3], the presence of the second β-(1 → 4)-linked glucose moiety evidently increases the relative amount of β-addition. The 2-deoxy-2-fluorocellobiose derivative **10** was treated with hydrazine acetate[26] to afford the hemiacetal derivative **12**, then converted to the acetylated 2,4-dinitrophenyl cellobioside **13** by reaction with FDNB[27]. Deacetylation with hydrogen chloride in methanol[28] gave the deprotected product **14**.

2,4-Dinitrophenyl 2-deoxy-2-fluoro-β-cellobioside **14** was found to be an effective inactivator of the exoglucanase from *Cellulomonas fimi*. This is the first

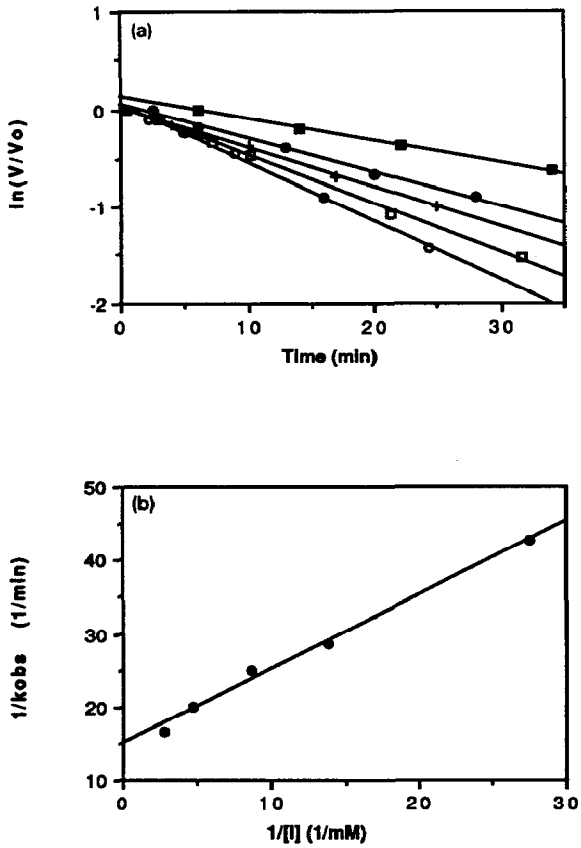
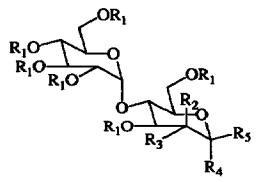
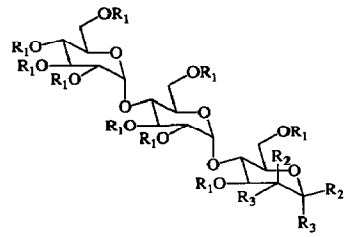


Fig. 1. Inactivation of *C. fimi* exoglucanase by 2,4-dinitrophenyl 2-deoxy-2-fluoro-β-cellobioside **14**. (a) Semi-logarithmic plot of residual activity versus time at the following inactivator concentrations: ■, 0.036; ●, 0.073; +, 0.12; □, 0.22; ○, 0.36 mM. (b) Double-reciprocal plot of first-order rate constants from (a).

reported instance of a disaccharide compound, an analog of the enzyme's normal substrate, acting as a mechanism-based inactivator of a cellulase (Fig. 1). Values of K_i and k_i were found to be 0.11 mM and 6.7×10^{-2} min^{-1}, respectively. We have previously reported the inactivation of this exoglucanase by 2,4-dinitrophenyl 2-deoxy-2-fluoro-β-D-glucopyranoside and identified the active-site nucleophile involved in catalysis as Glu-274[29]. However, inactivation of the enzyme by the 2-deoxy-2-fluoroglucoside is very slow ($k_i = 2.5 \times 10^{-4}$ versus $k_i = 6.7 \times 10^{-2}$ min^{-1} for the 2-deoxy-2-fluorocellobioside). In terms of k_i/K_i, the 2-deoxy-2-fluorocellobioside is 11 000 times more effective than the 2-deoxy-2-fluoroglucoside (6.09×10^{-1} versus 5.56×10^{-5} min^{-1} mM^{-1}). These results are consistent with the greater substrate activity of cellobiosides than glucosides and they reflect the fact that the enzyme is able to utilize the additional binding energy derived from binding of the second glucose moiety to increase turnover numbers.





	R_1	R_2	R_3	R_4	R_5		R_1	R_2	R_3
15	Ac	H	F	OAc	H	**21**	Ac	H	F
16	Ac	F	H	H	OAc	**22**	Ac	F	H
17	Ac	H	F	F	H	**23**	H	H	F
18	Ac	F	H	H	F				
19	H	H	F	F	H				

Ac = acetyl
DNP = 2,4-dinitrophenyl

Addition of acetyl hypofluorite to 3,6,2′,3′,4′,6′-hexa-*O*-acetylmaltal[21], an α-(1 → 4)-linked disaccharide derivative, in $CFCl_3$ at 0°C in the usual manner gave the per-*O*-acetylated 2-deoxy-2-fluoro-α-maltoside **15** and the β-addition product **16**, identified by their ^{19}F and ^{1}H NMR spectra, in a 2:1 ratio. The anomeric proton of **15** exhibited an extremely small coupling to the equatorial C-2 fluorine and a 3.9-Hz coupling to H-2 while that of **16** coupled strongly to the axial fluorine at C-2 ($J_{F,1}$ 18.7 Hz), as expected. Cooling to −23 or −78°C increased the ratio of α/β addition to 3 or 6 to 1, respectively. These ratios are similar to those observed for addition of acetyl hypofluorite to the β-(1 → 4)-linked, but otherwise identical disaccharide 3,6,2′,3′,4′,6′-hexa-*O*-acetylcellobial (Table I). Apparently, the presence of the additional sugar at C-4 influences the stereochemistry of addition to the glycal, but the anomeric configuration of the 1 → 4 linkage has little effect on the α/β ratio.

Direct addition of fluorine to these maltooligosaccharide glycals was also investigated since such reactions should give rise to 2-deoxy-2-fluoroglycosyl fluorides directly. Monosaccharide derivatives of this type have been shown previously[30] to act as excellent mechanism-based inactivators of glycosidases, the

TABLE I

α/β Ratios of acetyl hypofluorite addition to oligosaccharide glycals [a]

Product	Addition ratio (α/β)	Reaction temperature (°C)
10 and **11** [b]	1.5	0
	5	−78
15 and **16**	2	0
	3	−23
	6	−78

[a] Conditions as described in text. All fluorinations with acetyl hypofluorite in $CFCl_3$, except as noted.
[b] In 10:1 $CFCl_3$–CH_3CN.

anomeric fluoride serving as a good leaving group. This approach was therefore chosen for the synthesis of the 2-deoxy-2-fluoro-α-maltosyl **19** and α-maltotriosyl fluorides **23**, promising candidates as potential inactivators of "retaining" α-glycosidases acting on oligosaccharide substrates. Per-*O*-acetylated maltal and maltotrial were reacted with fluorine in $CFCl_3$ in the usual manner to afford mixtures of the corresponding 2-deoxy-2-fluoroglycosyl fluorides, **17** and **18**, and **21** and **22**, identified by their ^{19}F NMR spectra. Again, the presence of additional (1 → 4)-linked sugar(s) decreased the ratio of α/β addition in comparison with the analogous monosaccharide, the ratio of α/β addition being 1.3 : 1 and 1.1 : 1 for the maltal and maltotrial peracetates, respectively, versus almost 3 : 1 for tri-*O*-acetylglucal under identical conditions[3]. The acetylated 2-deoxy-2-fluoro-α-maltosyl and α-maltotriosyl fluorides **17** and **21** were purified by flash chromatography and deacetylated with sodium methoxide in methanol to give **19** and **23**.

Oligosaccharides **19** and **23** were found to be slow substrates of human pancreatic alpha-amylase and rabbit muscle glycogen debranching enzyme, respectively. The kinetic parameters for **19** with human pancreatic alpha-amylase were determined by monitoring release of fluoride with a fluoride ion electrode. Hydrolysis of the 2-deoxy-2-fluoro maltoside **19** obeyed Michaelis–Menten kinetics at lower concentrations of substrate but showed higher than expected rates of fluoride release at higher substrate concentrations. This behavior is likely due to breakdown of the glycosyl–enzyme intermediate via transglycosylation to a second substrate molecule rather than by hydrolysis. Such behavior is quite common among "retaining" enzymes[31]. Analysis of the reaction corresponding to the lower hydrolysis rate yielded values of $k_{cat} = 0.17\ s^{-1}$ and $K_m = 4.7$ mM. Comparison of these values with those of the parent compound, α-maltosyl fluoride ($k_{cat} = 443\ s^{-1}$ and $K_m = 4.5$ mM) reveals a 3000-fold reduction in k_{cat} upon substitution of the fluorine at C-2, presumably due to destabilization of electron-deficient transition states involved in the enzymic reaction. Similar results (not shown) were obtained for the 2-deoxy-2-fluoromaltotrioside **23** with glycogen debranching enzyme.

CONCLUSIONS

2,4-Dinitrophenyl 2-deoxy-2-fluoro-β-cellobioside **14** was found to be an effective inactivator of an exoglucanase from *C. fimi*, the first instance of a disaccharide mechanism-based cellulase inactivator. The failure of the 2-deoxy-2-fluoro-α-maltosyl and maltotriosyl fluorides **19** and **23** to inactivate alpha-amylase or glycogen debranching enzyme, respectively, is perhaps not unexpected. While 2-deoxy-2-fluoroglycosides with excellent leaving groups are inactivators of several "retaining" glycosidases, perusal of the list of eleven different glycosidases tested with their corresponding 2-deoxy-2-fluoroglycosyl fluorides[30] reveals that this strategy is highly successful for β-glycosidases, working on all those tested. However, it is quite unimpressive for α-glycosidases, working only partially in two cases and

failing in the other three. Thus the results on human alpha-amylase and rabbit muscle glycogen debranching enzyme, both of which are "retaining" enzymes[32,33], would appear to be consistent with these previous findings.

Acetyl hypofluorite is a versatile fluorinating agent for the syntheses of 2,2-disubstituted monosaccharides and 2-deoxy-2-fluorooligosaccharides from the corresponding glycals. Further, the addition of fluorine to oligosaccharide glycal derivatives to give 2-deoxy-2-fluoro-α-maltosyl and α-maltotriosyl fluorides was demonstrated. Although the syntheses of radiolabelled compounds was beyond the scope of this study, these fluorinations may be readily adapted to the syntheses of ^{18}F-labelled compounds with potential utility as radiotracers using positron emission tomography (PET).

EXPERIMENTAL

General methods and materials.—Melting points (mp) were determined on a Laboratory Devices Mel-temp II melting-point apparatus, and are uncorrected. Solvents and reagents used were either reagent grade, certified, or spectral grade. Reactions were monitored by thin layer chromatography (TLC) using Merck Kieselgel 60 F_{254} analytical plates. Compounds were detected (where possible) under UV light or by charring with 10% H_2SO_4 in MeOH. Flash chromatography was performed using silica columns of Kieselgel 60 (180–230 mesh). ^{1}H NMR spectra were recorded on 200-MHz Bruker AC-200 or 400-MHz WH-400 spectrometers. Chemical shifts (δ) recorded for solutions in $CDCl_3$ or D_2O were measured against internal $(CH_3)_4Si$ or external 4,4-dimethyl-4-silapentane-1-sulphonate, respectively. ^{19}F NMR spectra were recorded on a 200-MHz Bruker AC-200 spectrometer, chemical shifts (δ) are quoted relative to $CFCl_3$ and spectra are ^{1}H decoupled unless otherwise specified. Trifluoroacetic acid was used as an external standard. Desorption chemical-ionization high-resolution mass spectra (HRMS) generated with ammonia as the reactive gas were recorded on a Delsi Nermag R10-10C mass spectrometer. Cellobiose octaacetate and maltotriose were obtained from the Aldrich and Sigma Chemical companies, respectively.

General fluorination procedure with acetyl hypofluorite or fluorine.—The glycal ($\sim$ 0.1–1 mmol) was dissolved in 10 mL of CH_3CN or $CFCl_3$, or in a mixture of these solvents as required to dissolve the glycal. A mixture of F_2 diluted with Ne was passed through a column of potassium acetate and bubbled through the solution at 150 mL/min at the temperature specified. Fluorinations in $CFCl_3$ were carried out at 0°C, unless otherwise specified, when appropriate cooling baths were employed to achieved the desired temperatures. When TLC indicated that no starting material remained, the solvent was evaporated in vacuo and the product(s) purified by flash chromatography. Fluorinations using only F_2 were carried out in an identical manner, except that the potassium acetate column was omitted and the F_2 passed directly through the glycal solution.

2(R)-2-Acetoxy-1,3,4,6-tetra-O-acetyl-2-fluoro-α-D-arabino-hexopyranose (**1**).—A solution of 2-acetoxy-3,4,6-tri-*O*-acetyl-D-glucal[16] (107 mg, 0.324 mmol) in 10:1 $CFCl_3$–CH_3CN was reacted with AcOF at 0°C according to the general procedure. Flash chromatography (2:1 petroleum ether–EtOAc) afforded **1** (30 mg, 23%) as a colourless oil; ^1H NMR ($CDCl_3$): δ 6.95 (s, 1 H, H-1), 5.57 (dd, 1 H, $J_{3,F}$ 5.65, $J_{3,4}$ 10.0 Hz, H-3), 5.16 (dd, $J_{4,5}$ 10.0 Hz, H-4), 4.10–3.97 (m, 3 H, H-5, H-6a,6b), and 2.21–2.00 (5 s, 15 H, 5 OAc); ^{19}F NMR ($CDCl_3$): δ −133.99 (s, F-2e); HRMS calcd for $C_{16}H_{21}FO_{11}$: $(M + NH_4^+)$, 426.1412; found: $(M + NH_4^+)$, 426.1412.

2(R)-2-Acetoxy-1,3,4,6-tetra-O-acetyl-2-fluoro-α-D-lyxo-hexopyranose (**2**).—A solution of 2-acetoxy-3,4,6-tri-*O*-acetyl-D-galactal[16] (212 mg, 0.642 mmol) in 5:1 $CFCl_3$–CH_3CN was treated with AcOF at 0°C according to the general procedure. Flash chromatography (2:1 petroleum ether–EtOAc) afforded **2** (63 mg, 24%) as a colourless oil; ^1H NMR ($CDCl_3$): δ 7.02 (s, 1 H, H-1), 5.40 (m, 2 H, $J_{3,F}$ 6.0 Hz, H-3, H-4), 4.31 (t, 1 H, $J_{5,6a,b}$ 6.4 Hz, H-5), 4.10–4.0 (m, 2 H, H-6a,6b), 2.14 (2 s, 6 H, 2 OAc), 2.10, 2.05, and 1.97 (3 s, 9 H, 3 OAc); ^{19}F NMR ($CDCl_3$): δ −133.98 (s, F-2e); HRMS calcd for $C_{16}H_{21}FO_{11}$: $(M + NH_4^+)$, 426.1412; found: $(M + NH_4^+)$, 426.1404.

3,4,6-Tri-O-acetyl-2-fluoro-D-galactal (**3**).—3,4,6-Tri-*O*-acetyl-2-deoxy-2-fluoro-α-D-galactopyranosyl fluoride[17] (1.00 g, 3.22 mmol) was dissolved in 45% HBr–HOAc (5 mL) containing three drops of Ac_2O and stirred at room temperature for 18 h. The mixture was dissolved in CH_2Cl_2, washed successively with water, satd $NaHCO_3$ solution and water, and dried ($MgSO_4$). Evaporation of solvent in vacuo afforded an oil which was dissolved in CH_3CN (30 mL), together with Et_3N (4.9 mL, 35 mmol) and refluxed for 18 h. The mixture was concentrated to a gum and purified by flash chromatography (4:1 petroleum ether–EtOAc) to give **3** (0.47 g, 50% from the fluoride); ^1H NMR ($CDCl_3$): δ 6.68 (d, 1 H, $J_{1,F}$ 4.4 Hz, H-1), 5.82 (d, 1 H, $J_{3,4}$ 5.2 Hz, H-3), 5.38 (m, 1 H, H-4), 4.3–4.1 (m, 3 H, H-5, H-6a,6b), 2.08 (s, 3 H, 1 OAc), and 2.02 (2 s, 6 H, 2 OAc); ^{19}F NMR ($CDCl_3$): δ −168.70 (s, F-2). Anal. Calcd. for $C_{12}H_{15}FO_7$: C, 49.70; H, 5.20. Found: C, 49.67; H, 5.30.

3,4,6-Tri-O-acetyl-2-deoxy-2,2-difluoro-α-D-lyxo-hexopyranosyl bromide (**5**).—A solution of the 2-fluorogalactal **3** (88 mg, 0.303 mmol) in $CFCl_3$ was treated with AcOF at 0°C according to the general procedure. Flash chromatography (2:1 petroleum ether–EtOAc) afforded the 2-deoxy-2,2-difluoro-*lyxo*-hexose **4** (87 mg, 78%) as an amorphous solid; ^1H NMR ($CDCl_3$): δ 6.19 (d, 1 H, $J_{1,Fa}$ 6.0 Hz, H-1), 5.42 (m, 1 H, H-4), 5.39 (ddd, 1 H, $J_{3,Fa}$ 20.0, $J_{3,Fe}$ 7.6, $J_{3,4}$ 3.6 Hz, H-3), 4.30 (m, 1 H, H-5), 4.08–4.00 (m, 2 H, H-6a,6b), and 2.15, 2.09, 2.06, 1.97 (4 s, 12 H, 4 OAc); ^1H coupled-^{19}F NMR ($CDCl_3$): δ −118.91 (ddd, $J_{Fa,Fe}$ 257, $J_{Fa,1}$ 6.0, $J_{Fa,3}$ 20.0 Hz, F*a*-2) and −120.92 (dd, $J_{Fe,Fa}$ 257, $J_{Fe,3}$ 7.6 Hz, F*e*-2). The *lyxo*-hexose **4** (87 mg, 0.236 mmol) was dissolved in 45% HBr–HOAc (5 mL) containing three drops Ac_2O and stirred for 8 days at room temperature. Dichloromethane was added and the solution washed successively with water, satd $NaHCO_3$ solution and water, and dried ($MgSO_4$). Evaporation of solvent in vacuo gave a gum which was purified by flash chromatography (4:1 petroleum ether–EtOAc) to afford **5** (31

mg, 34%) as a yellow oil; ^{1}H NMR ($CDCl_3$): δ 6.17 (d, 1 H, $J_{1,Fa}$ 6.8 Hz, H-1), 5.62 (m, 1 H, H-4), 5.37 (ddd, 1 H, $J_{3,Fa}$ 22.0, $J_{3,Fe}$ 6.6, $J_{3,4}$ 4.0 Hz, H-3), 4.29 (dt, 1 H, $J_{6,5}$ 7.0, $J_{5,4}$ 1.6 Hz, H-5), 3.2–3.1 (m, 2 H, H-6a,6b), and 2.15, 2.11, 2.06 (3 s, 9 H, 3 OAc); ^{1}H coupled–^{19}F NMR ($CDCl_3$): δ −119.01 (ddd, $J_{Fa,Fe}$ 257, $J_{Fa,3}$ 22, $J_{Fa,1}$ 6.8 Hz, F*a*-2) and −121.29 (dd, $J_{Fe,3}$ 6.6, $J_{Fe,Fa}$ 257 Hz, F*e*-2); HRMS calcd for $C_{12}H_{15}BrF_2O_7$: (M + H$^+$), 389.0048; found (M + H$^+$), 389.0070. On scale-up, **5** was obtained in 47% overall yield from the per-*O*-acetylated 2-fluorogalactal **3**.

*2,4-Dinitrophenyl 3,4,6-tri-*O*-acetyl-2-deoxy-2,2-difluoro-α-*D-arabino-*hexopyranoside* (**8**).—A solution of 3,4,6-tri-*O*-acetyl-2-fluoro-D-glucal[34] (130 mg, 0.45 mmol) in $CFCl_3$ was treated with AcOF at 0°C according to the general procedure. The resulting mixture was partially purified by flash chromatography (1:1 petroleum ether–EtOAc) to afford a syrup, containing an anomeric mixture (largely α) of 1,3,4,6-tri-*O*-acetyl-2-deoxy-2,2-difluoro-D-arabino-hexopyranose **6**, which resisted further purification. The syrup was treated with hydrazine acetate[26] (40 mg, 0.43 mmol) in DMF (5 mL) for 12 h at room temperature. Evaporation of solvent in vacuo, followed by flash chromatography (1:1 petroleum ether–EtOAc) gave the hemiacetal **7** (77 mg, 52% from the per-*O*-acetylated 2-fluoroglucal) as a colourless oil; ^{1}H NMR ($CDCl_3$): δ 5.62 (ddd, 1 H, $J_{3,Fa}$ 20.0, $J_{3,4}$ 9.0, $J_{3,Fe}$ 4.3 Hz, H-3), 5.26 (d, 1 H, $J_{1,Fa}$ 4.5 Hz, H-1), 5.23 (t, 1 H, $J_{4,3}$ 10, $J_{4,5}$ 10 Hz, H-4), 4.40–4.12 (m, 3 H, H-5, H-6a,6b), 2.14, 2.10, and 2.07 (3 s, 9 H, 3 OAc); ^{19}F NMR ($CDCl_3$); δ −120.74 (d, $J_{Fe,Fa}$ 253 Hz, F*e*-2) and −122.71 (d, $J_{Fa,Fe}$ 253 Hz, F*a*-2). The hemiacetal **7** (30 mg, 0.09 mmol) was dissolved in dry DMF (2 mL) and treated with 1-fluoro-2,4-dinitrobenzene (FDNB, 17 mg, 0.09 mmol) and 1,4-diazabicyclo[2.2.2]octane (DABCO, 23 mg, 0.25 mmol)[27]. The mixture was stirred under N_2 at 70°C for 2 h over molecular sieves. The suspension was filtered and solvent evaporated in vacuo. Since TLC analysis indicated that partial deacetylation had occurred, the resulting oil was stirred for 2 days in 1:1 pyridine–Ac_2O at room temperature. The solvent was removed in vacuo and the residue dissolved in $CHCl_3$, washed with 5% $NaHCO_3$ and dried ($MgSO_4$). The glycoside **9** was crystallized from EtOH and recrystallized from the same solvent to afford yellowish crystals (10 mg, 22%); mp 218–220°C; ^{1}H NMR ($CDCl_3$) δ 8.80 (d, 1 H, $J_{3',5'}$ Hz, H-3'), 8.42 (dd, 1 H, $J_{5',6'}$ 9, $J_{5',3'}$ 3 Hz, H-5'), 7.42 (d, 1 H, $J_{6',5'}$ 9 Hz, H-6'), 581–5.61 (m, 2 H, H-1, H-3), 5.29 (t, 1 H, $J_{4,3}$ 10, $J_{4,5}$ 10 Hz, H-4), 4.28–4.20 (m, 3 H, H-5, H-6a,b), 2.11, 2.13, and 2.00 (3 s, 9 H, 3 OAc); ^{1}H coupled–^{19}F NMR ($CDCl_3$): δ −119.95 (dd, $J_{Fe,Fa}$ 260, $J_{Fe,3}$ 9.5 Hz, F*e*-2), −121.36 (ddd, $J_{Fa,Fe}$ 260, $J_{Fa,3}$ 12.6, $J_{Fa,1}$ 3.2 Hz, F*a*-2). Anal. Calcd for $C_{18}H_{18}F_2N_2O_{12}$: C, 43.91; H, 3.68; N, 5.69. Found: C, 43.51; H, 3.80; N, 5.40.

*2(*R*)-2-Acetamido-2-acetoxy-3,4,6-tri-*O*-acetyl-α-*D-arabino-*hexopyranosyl fluoride* (**9**).—A solution of 2-acetamido-3,4,6-tri-*O*-acetyl-D-glucal[15] (26 mg, 0.079 mmol) in CH_3CN was treated with AcOF at room temperature according to the general procedure. Flash chromatography (7:3 diethyl ether–EtOAc) gave **9** (9.5 mg, 30%) as an oil; ^{1}H NMR ($CDCl_3$): δ 6.83 (d, 1 H, $J_{1,Fa}$ 50.9 Hz, H-1), 6.54 (br d, 1 H, $J_{NH,F}$ 3.8 Hz, NH), 5.62 (dd, 1 H, $J_{3,4}$ 10.0, $J_{3,Fa}$ 1.8 Hz, H-3), 5.18 (dd, 1

H, $J_{4,5}$ 10.0 Hz, H-4), 4.4–4.1 (m, 3 H, H-5, H-6a,6b), and 2.15–2.00 (5 s, 15 H, 4 OAc and NAc); ^{19}F NMR ($CDCl_3$): δ −150.97 (s, F*a*-1); HRMS calcd for $C_{16}H_{22}FNO_{10}$: (M^+), 407.1228; found: (M^+), 407.1202.

1,3,6,2',3',4',6'-Hepta-O-acetyl-2-deoxy-2-fluoro-α-cellobiose (**10**).—3,6,2',3',4',6'-Hexa-*O*-acetylcellobial[20] (80 mg, 0.14 mmol) was dissolved in 10:1 $CFCl_3$–CH_3CN and treated with AcOF at 0°C according to the general procedure to afford a 1.5:1 mixture of **10** and the β-addition product **11**, identified by their ^{19}F NMR spectra. Selective crystallization from diethyl ether–$CHCl_3$, and recrystallization from the same solvent gave pure **10** as white crystals (17 mg, 0.026 mmol, 19%); mp 193–194°C; ^{1}H NMR ($CDCl_3$): δ 6.35 (d, $J_{1,2}$ 4 Hz, H-1), 5.60–3.45 (m, H-2,3 and H-1'-6'), and 2.00–2.15 (7 s, 7 OAc); ^{1}H coupled–^{19}F NMR ($CDCl_3$): δ −201.8 (dd, $J_{2,F}$ 50, $J_{3,F}$ 12 Hz, F*e*-2); HRMS calcd for $C_{26}H_{36}FO_{17}$: ($M + H^+$), 639.1936; found: ($M + H^+$), 639.1892. **11** (not isolated); ^{1}H coupled–^{19}F NMR ($CDCl_3$); δ −219.4 (ddd, $J_{1,F}$ 18, $J_{2,F}$ 50, $J_{3,F}$ 27 Hz, F*a*-2).

3,6,2',3',4',6'-Hexa-O-acetyl-2-deoxy-2-fluoro-cellobiose (**12**).—A mixture of the 2-deoxy-2-fluoro peracetate **10** (200 mg, 0.31 mmol) and hydrazine acetate (35 mg, 0.38 mmol) in DMF (1 mL) was stirred at 50°C until dissolved then stirred for 3 h at room temperature[26]. The mixture was dissolved in EtOAc, washed with NaCl solution and the solvent evaporated in vacuo, residual DMF being removed by several co-evaporations with toluene. The hemiacetal **12** was recrystallized from $CHCl_3$–petroleum ether as a white solid (139 mg, 71%), mp 213–215°C; ^{1}H NMR ($CDCl_3$): δ 5.60–3.40 (m, H-1-6 and H-1'-6'), and 1.95–2.05 (6 s, 6 OAc). ^{1}H coupled–^{19}F NMR ($CDCl_3$): δ −199.48 [ddd, $J_{2,F}$ 50, $J_{3,F}$ 12, $J_{1,F}$ 2.0 Hz, F-2 (β anomer)], and −200.10 [dd, $J_{2,F}$ 50, $J_{3,F}$ 12 Hz, F-2 (α anomer)]; HRMS calcd for $C_{24}H_{34}FO_{16}$: ($M + H^+$), 597.1831; found: ($M + H^+$), 597.1838.

2,4-Dinitrophenyl 3,6,2',3',4',6'-hexa-O-acetyl-2-deoxy-2-fluoro-β-cellobioside (**13**).—A solution of the hemiacetal **12** (130 mg, 0.22 mmol) and DABCO (72 mg, 0.64 mmol) in DMF (3 mL) was stirred over molecular sieves for 3 h, FDNB (48 mg, 0.26 mmol) was added and the mixture was stirred at room temperature for 24 h[27]. The sieves were removed by gravity filtration, washed with $CHCl_3$, and the filtrate evaporated in vacuo to yield a gum which was dissolved in $CHCl_3$, washed with satd $NaHCO_3$ solution, and dried ($MgSO_4$). Purification by flash chromatography (1:1 EtOAc–petroleum ether) and recrystallization from EtOAc–petroleum ether yielded compound **13** (55 mg, 33%); mp 182–183°C; ^{1}H NMR ($CDCl_3$); δ 8.75 (d, $J_{3'',5''}$ 4 Hz, H-3''), 8.45 (dd, $J_{5'',3''}$ 4, $J_{5'',6''}$ 9 Hz, H-5''), 7.40 (d, $J_{6'',5''}$ 9 Hz, H-6''), 5.45–3.65 (m, H-1-6 and H-1'-6'), and 2.00–2.15 (6 s, 6 OAc); ^{19}F NMR ($CDCl_3$): δ −197.12 (s, F-2). Anal. Calcd for $C_{30}H_{35}FN_2O_{20}$: C, 47.24; H, 4.59; N, 3.67. Found: C, 46.95; H, 4.67; N, 3.62.

2,4-Dinitrophenyl 2-deoxy-2-fluoro-β-cellobioside (**14**).—The acetylated cellobioside **13** (48 mg, 0.063 mmol) was suspended in MeOH (3 mL) and AcCl (0.3 mL) and stirred at 4°C for 24 h[28]. Evaporation of the solvent in vacuo yielded a gum which was triturated with anhyd diethyl ether. Compound **14** was crystallized from EtOH as a white solid (11 mg, 35%); mp 178–179°C; ^{1}H NMR (CD_3OD): δ 8.75

(d, $J_{3'',5''}$ 2 Hz, H-3''), 8.50 (dd, $J_{5'',3''}$ 2, $J_{5'',6''}$ 9 Hz, H-5''), 7.65 (d, $J_{6'',5''}$ 9 Hz, H-6''), 5.65 (d, $J_{1,2}$ 9 Hz, H-1), 3.20–5.10 (m, H-2-6 and H-1'-6'). ^{1}H coupled–^{19}F NMR (CD_3OD): δ −198.41 (dd, $J_{F,3}$ 16, $J_{F,2}$ 50 Hz, F-2). Anal. Calcd for $C_{18}H_{23}FN_2O_{14}$: C, 42.35; H, 4.51; N, 5.49. Found: C, 41.73; H, 4.67; N, 5.19.

2-Deoxy-2-fluoro-α-maltosyl fluoride (**19**).—A solution of maltal peracetate[21] (500 mg, 0.89 mmol) in $CFCl_3$ was reacted with F_2 at −78°C according to the general procedure to give a 1.3:1 mixture of the 2-deoxy-2-fluoromaltose **17** and the β-addition product **18**, identified by their ^{19}F NMR spectra. Flash chromatography (1:1:1 EtOAc–petroleum ether–$CHCl_3$) afforded the peracetate **17** (108 mg, 20%) as a colourless gum; ^{1}H NMR ($CDCl_3$): δ 5.68 (dd, 1 H, $J_{1,F1}$ 53.4, $J_{1,2}$ 2.6 Hz, H-1), and 2.14–1.95 (6 s, 6 OAc); ^{19}F NMR ($CDCl_3$): δ −151.2 (d, $J_{F1,F2}$ 18.8 Hz, F-1), −205.5 (d, $J_{F2,F1}$ 18.8 Hz, F-2). **18** (not isolated); ^{19}F NMR ($CDCl_3$): δ −145.3 [(d, $J_{F1,F2}$ 13.8 Hz, F-1), −219.7 (d, $J_{F2,F1}$ 13.8 Hz, F-2)]. To a solution of **17** (100 mg, 0.17 mmol) in dry MeOH (10 mL) was added NaOMe (3 mL, 1.77 mmol) and the mixture was stirred under N_2 for 1 h. The mixture was neutralized with Dowex AG-50W X2(H^+) resin and solvent was evaporated in vacuo. Flash chromatography (5:2:1 EtOAc–EtOH–water) afforded **19** (53 mg, 90%) as a colourless gum. ^{1}H NMR ($CDCl_3$): δ 5.85 (dd, 1 H, $J_{1,F1}$ 53.9, $J_{1,2}$ 2.4 Hz, H-1); ^{19}F NMR ($CDCl_3$): δ −149.1 (d, $J_{F1,F2}$ 20.0 Hz, F-1), −204.6 (d, $J_{F2,F1}$ 20.0 Hz, F-2). Anal. Calcd. for $C_{12}H_{20}F_2O_9$: C, 41.62; H, 5.82. Found: C, 41.28; H, 5.96.

3,6,2',3',6',2'',3'',4'',6''-Nona-O-acetylmaltotrial (**20**).—To a solution of maltotriose peracetate (1.38 g, 1.43 mmol) in glacial HOAc (10 mL) was added 45% HBr in HOAc (2.2 mL) and the mixture stirred under N_2 for 1 h at 0°C. The mixture was dissolved in cold $CHCl_3$, washed successively with water, satd $NaHCO_3$ solution and water, and dried ($MgSO_4$). Evaporation of solvent in vacuo gave a gum which was triturated in cold petroleum ether to form a white powder. This was dissolved in 1:1 water–HOAc (15 mL), activated zinc (3.3 g, 50.5 mmol) added, and the mixture stirred overnight at 0°C. The mixture was dissolved in cold $CHCl_3$ and washed successively with water, satd $NaHCO_3$ solution and water, and dried ($MgSO_4$). Flash chromatography (1:2 petroleum ether–EtOAc) yielded pure **20** (0.51 g, 42%) as a colourless gum; ^{1}H NMR ($CDCl_3$): δ 6.44 (dd, 1 H, $J_{1,2}$ 6.0, $J_{1,3}$ 1.0 Hz, H-1), and 2.15–1.95 (9 s, 9 OAc); HRMS calcd for $C_{36}H_{52}NO_2$: ($M + NH_4^+$), 866.2930; found: ($M + NH_4^+$), 866.2881.

2-Deoxy-2-fluoro-α-maltotriosyl fluoride (**23**).—A solution of **20** (344 mg, 0.405 mmol) in $CFCl_3$ was treated with F_2 at −78°C according to the general procedure to afford a 1.1:1 mixture of the 2-deoxy-2-fluoro maltotriose **21** and the β-addition product **22**, identified by their ^{19}F NMR spectra. Flash chromatography (2:1:1 EtOAc–petroleum ether–$CHCl_3$) yielded **21** (133 mg, 37%) as a colourless gum; ^{1}H NMR ($CDCl_3$): δ 5.68 (dd, 1 H, $J_{1,F1}$ 52.7, $J_{1,2}$ 2.6 Hz, H-1), and 2.15–1.95 (9 s, 9 OAc); ^{19}F NMR ($CDCl_3$): δ −151.2 (d, $J_{F1,F2}$ 18.8 Hz, F-1), −205.3 (d, $J_{F2,F1}$ 18.8 Hz, F-2). **22** (not isolated); ^{19}F NMR ($CDCl_3$): δ −144.9 (d, $J_{F1,F2}$ 14.2 Hz, F-1), −219.4 (d, $J_{F2,F1}$ 14.2 Hz, F-2). To a solution of **21** (106 mg, 0.12 mmol) in dry MeOH (10 mL) was added NaOMe (3 mL, 1.76 mmol) and the reaction was

stirred for 1 h under N_2. Dowex AG-50W X2 (H^+) resin was added to neutralize the solution and the solvent was evaporated in vacuo. Flash chromatography (5:2:1 EtOAc–EtOH–water) yielded pure **23** (30 mg, 49%) as a colourless gum; ^1H NMR ($CDCl_3$); δ 5.85 (dd, 1 H, $J_{1,F1}$ 53.5, $J_{1,2}$ 2.3 Hz, H-1); ^{19}F NMR ($CDCl_3$): δ −150.3 (d, $J_{F1,F2}$ 19.8 Hz, F-1), −204.6 (d, $J_{F2,F1}$ 19.8 Hz, F-2). Anal. Calcd for $C_{18}H_{30}F_2O_{14} \cdot H_2O$: C, 41.06; H, 6.13. Found: C, 40.80; H, 6.16.

Enzyme kinetics.—Kinetic parameters for the inactivation of *C. fimi* exoglucanase by 2,4-dinitrophenyl 2-deoxy-2-fluoro-β-cellobioside were determined by incubation of the enzyme in the presence of bovine serum albumin (1 mg/mL) and varying concentrations of the inactivator in sodium phosphate buffer (50 mM, pH 7.0) at 37°C. Aliquots were removed at different time intervals and diluted into reaction cells containing a large volume of substrate (2,4-dinitrophenyl β-D-glucopyranoside or 2,4-dinitrophenyl β-cellobioside) at saturating concentrations. The residual enzymic activity was then determined from the rate of hydrolysis of the substrate which is directly proportional to the amount of active enzyme. The inactivation was monitored until 80–90% of enzymic activity was depleted. From the slope of the plot of the natural logarithm of the residual activity versus time, pseudo-first-order rate constants (k_{obs}) at each inactivator concentration were calculated. Fitting values of k_{obs} to a nonlinear form of the Michaelis–Menten equation using a weighted nonlinear regression program (*GraFit*)[35] afforded values for K_i and k_i.

Kinetic parameters for reaction of 2-deoxy-2-fluoro-α-maltosyl fluoride and α-maltotriosyl fluoride with alpha-amylase and glycogen debranching enzyme, respectively, were determined by monitoring fluoride release. Enzyme was added to cells containing several different substrate concentrations in phosphate buffer (20 mM sodium phosphate, 25 mM NaCl, pH 6.9) incubated at 30°C and fluoride release was monitored using an Orion 96-09 combination fluoride-ion electrode. Initial rates were determined and the kinetic constants, k_{cat} and K_m were determined by fitting the rates to the Michaelis–Menten equation using the *GraFit* nonlinear regression program described above.

REFERENCES

1 M.J. Adam, *J. Chem. Soc., Chem. Commun.*, (1982) 730–731.
2 C.-Y. Shiue, J.S. Fowler, A.P. Wolf, D. Alexoff, and R.R. MacGregor, *J. Label. Comp. Radiopharm.*, 22 (1985) 503–508.
3 N. Satyamurthy, G.T. Bida, H.C. Padgett, and J.R. Barrio, *J. Carbohydr. Chem.*, 4 (1985) 489–512.
4 K. Dax and B. Glänzer, *Carbohydr. Res.*, 162 (1987) 13–22.
5 C.-Y. Shiue, P.A. Salvadori, A.P. Wolf, J.S. Fowler, and R.R. MacGregor, *J. Nucl. Med.*, 23 (1982) 899–903.
6 M. Diksic and D. Jolly, *Int. J. Appl. Radiat. Isot.*, 34 (1983) 893–896.
7 A. Dessinges, F.C. Escribano, G. Lukacs, A. Olesker, and T.T. Thang, *J. Org. Chem.*, 52 (1987) 1633–1644.
8 C. Bliard, P. Herczegh, A. Olesker, and G. Lukacs, *J. Carbohydr. Chem.*, 8 (1989) 103–113.
9 K. Bischofberger, R.H. Hall, A. Jordaan, and G.R. Woolard, *S. Afr. J. Chem.*, 33 (1980) 92–94.
10 J. Adamson, A.B. Foster, and J.H. Westwood, *Carbohydr. Res.*, 18 (1971) 345–347.

11 S.G. Withers and I.P. Street, *J. Am. Chem. Soc.*, 110 (1988) 8551–8553.
12 I.P. Street, J.B. Kempton, and S.G. Withers, *Biochemistry*, 31 (1992) 9970–9978.
13 J.D. McCarter, M.J. Adam, and S.G. Withers, *Biochem. J.*, 286 (1992) 721–727.
14 M. Namchuk and S.G. Withers, unpublished results.
15 N. Pravdic, I. Franjic-Mihalic, and B. Danilov, *Carbohydr Res.*, 45 (1975) 302–306.
16 R.L. Whistler and M.L. Wolfrom (Eds.), *Methods Carbohydr. Chem.* II, [104], New York (1963).
17 J.G. Shelling, D. Dolphin, P. Wirz, R.E. Cobbledick, and F.W.B. Einstein, *Carbohydr. Res.*, 132 (1984) 241–259.
18 R.U. Lemieux and A.R. Morgan, *Can. J. Chem.*, 43 (1965) 2214–2221.
19 J. Adamson and A.B. Foster, *Carbohydr. Res.*, 10 (1969) 517–523.
20 T. Kanda, C.F. Brewer, G. Okada and E.J. Hehre, *Biochemistry*, 25 (1986) 1159–1165.
21 E.J. Hehre, S. Kitahata, and C.F. Brewer, *J. Biol. Chem.*, 261 (1986) 2147–2153.
22 A. El-Laghdach, M.I. Matheu, S. Castillon, C. Bliard, A. Olesker, and G. Lukacs, *Carbohydr. Res.*, 233 (1992) c1–c3.
23 P.J. Card and G.S. Reddy, *J. Org. Chem.*, 48 (1983) 4734–4743.
24 P.W. Kent, R.A. Dwek, and N.F. Taylor, *Biochem. J.*, 121 (1971) 10P–11P.
25 A.B. Foster, R. Hems, and L.D. Hall, *Can. J. Chem.*, 48 (1970) 3937–3945.
26 G. Excoffier, D. Gagnaire, and J.-P. Utille, *Carbohydr. Res.*, 39 (1975) 368–373.
27 H.J. Koener, A.J. De Hok, C. Romer, and J.H. Van Boom, Recl. Trav. Chim. Pays-Bas, 99 (1980) 355–362.
28 F. Ballardie, B. Capon, D. Sutherland, D. Cocker, and M.L. Sinnott, *J. Chem. Soc.*, Perkin Trans. 1, (1973) 2418–2419.
29 D. Tull, S.G. Withers, N.R. Gilkes, D.G. Kilburn, R.A.J. Warren, and R. Aebersold, *J. Biol. Chem.*, 266 (1991) 15621–15625.
30 S.G. Withers, K. Rupitz, and I.P. Street, *J. Biol. Chem.*, 26 (1988) 7929–7932.
31 J.B. Kempton and S.G. Withers, *Biochemistry*, 31 (1992) 9961–9969.
32 W. Liu, N.B. Madsen, C. Braun, and S.G. Withers, *Biochemistry*, 30 (1991) 1419–1424.
33 C. Braun and S.G. Withers, unpublished results.
34 J. Adamson, A.B. Foster, and J.H. Westwood, *Carbohydr. Res.*, 18 (1971) 345–347.
35 R.J. Leatherbarrow, *GraFit Version* 2.0, Erithacus Software Ltd., Staines, UK, 1990.