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The chemical synthesis of 2-deoxy-2-fluorodisaccharide probes of the hen egg white lysozyme mechanism

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Abstract—2,4-Dinitrophenyl 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-2-deoxy-2-fluoro-β-D-glucopyranoside (GN2FG-DNP) and 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-2-deoxy-2-fluoro-β-D-glucopyranosyl fluoride (GN2FG-F) were prepared using a divergent synthetic approach involving 10 steps. The key steps involved the preparation of 1-*O*-acetyl-3,6-di-*O*-benzyl-2-deoxy-2-fluoro-α/β-D-glucopyranose using Selectfluor™ in the presence of acetic acid and the subsequent glycosylation of this acceptor to generate the core 2-fluorodisaccharide. After further elaboration, the target molecules were obtained and tested as probes of the mechanism of hen egg white lysozyme (HEWL). Compound GN2FG-DNP is not a substrate for the enzyme while compound GN2FG-F is cleaved slowly with an apparent K_m greater than 5 mM and a second-order rate constant of $k_{cat}/K_m = 9.6\ s^{-1}M^{-1}$. Comparison of this value to that estimated for the hydrolysis of β-chitobiosyl fluoride by HEWL ($1200\ s^{-1}M^{-1}$) [Ballardie, F. W.; Capon, B.; Cuthbert, M. W.; Dearie, W. M. *Bioorg. Chem.* **1977**, *6*, 483–509] revealed a 126-fold rate decrease upon substitution of a fluorine group for the 2-acetamido group of β-chitobiosyl fluoride. This decrease resulted in the steady-state accumulation of an intermediate as visualized by mass spectrometry and the ultimate crystallographic determination of its structure [Vocadlo, D. J.; Davies, G. J.; Laine, R.; Withers, S. G. *Nature* **2001**, *412*, 835–838].

Keywords: Fluorine; Hen egg white lysozyme (HEWL); Catalytic mechanism; Mechanism-based inactivator; Glycosyl fluoride, inhibitor

1. Introduction

The catalytic mechanism of HEWL has been the subject of many studies. Despite having gained textbook familiarity, however, certain aspects of the mechanism have long remained contentious. Early studies confirmed the majority of the features of the catalytic mechanism proposed by Phillips and coworkers.[1] Yet the most contentious point, which has until recently[2] remained unclear, is the nature of the reaction intermediate. The original catalytic mechanism proposed by Phillips on the basis of model building studies, in conjunction with the three-dimensional structure determined by X-ray diffraction,[3] suggests that the intermediate is a long-lived metastable ion pair formed between the oxocarbenium ion and the enzymic carboxylate Asp-52 (Chart 1). The arguments against a covalent intermediate are based on several three-dimensional models of HEWL-product complexes.[1,4] According to these interpretations, the saccharide is positioned too far away from Asp-52, the orientation of Asp-52 is incorrect for it to act in a nucleophilic capacity, and the active site is too congested for a covalent intermediate to form.[4] A mechanism involving a long-lived ion-pair intermediate, however, is at odds with physical organic studies showing that in the presence of a negative ion there is essentially no barrier to the collapse of the glycosyl cation.[5–7] Additionally, α-deuterium kinetic isotope effects (KIE) measured using substrates for which the rate-determining step is breakdown of the intermediate provide evidence for a covalent intermediate. Such isotope effects have been carried out with several β-retaining glycosidases including, for example, the β-galactosidases from *E. coli*

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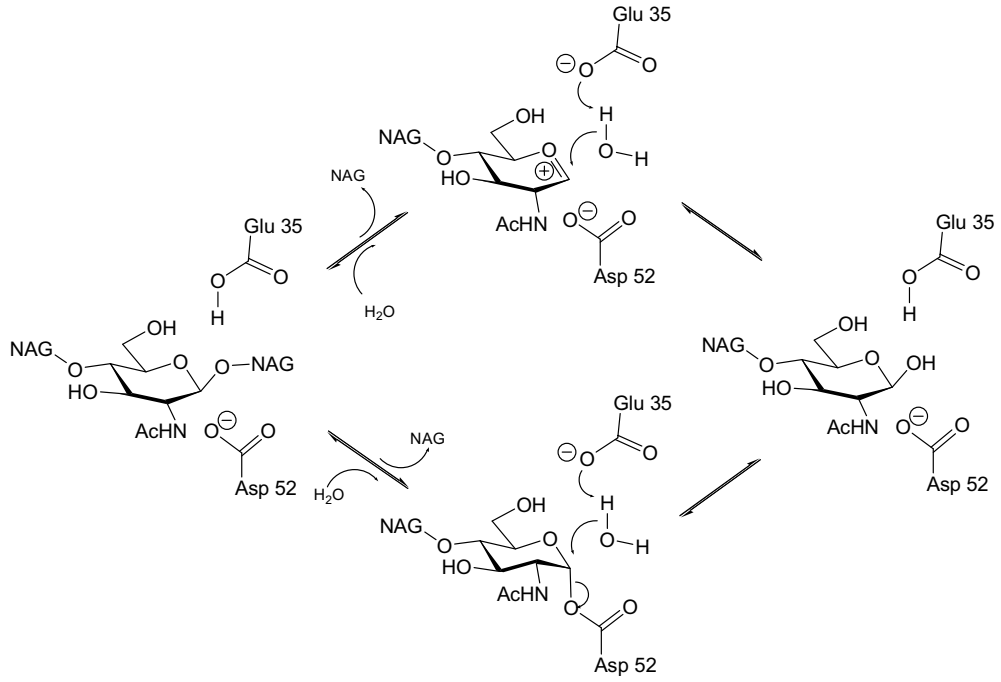


Chart 1. Possible mechanisms for hen egg white lysozyme. The upper path shows a mechanism involving a metastable oxocarbenium ion as proposed by Phillips and the lower path shows a covalent glycosyl-enzyme intermediate as first proposed by Koshland.

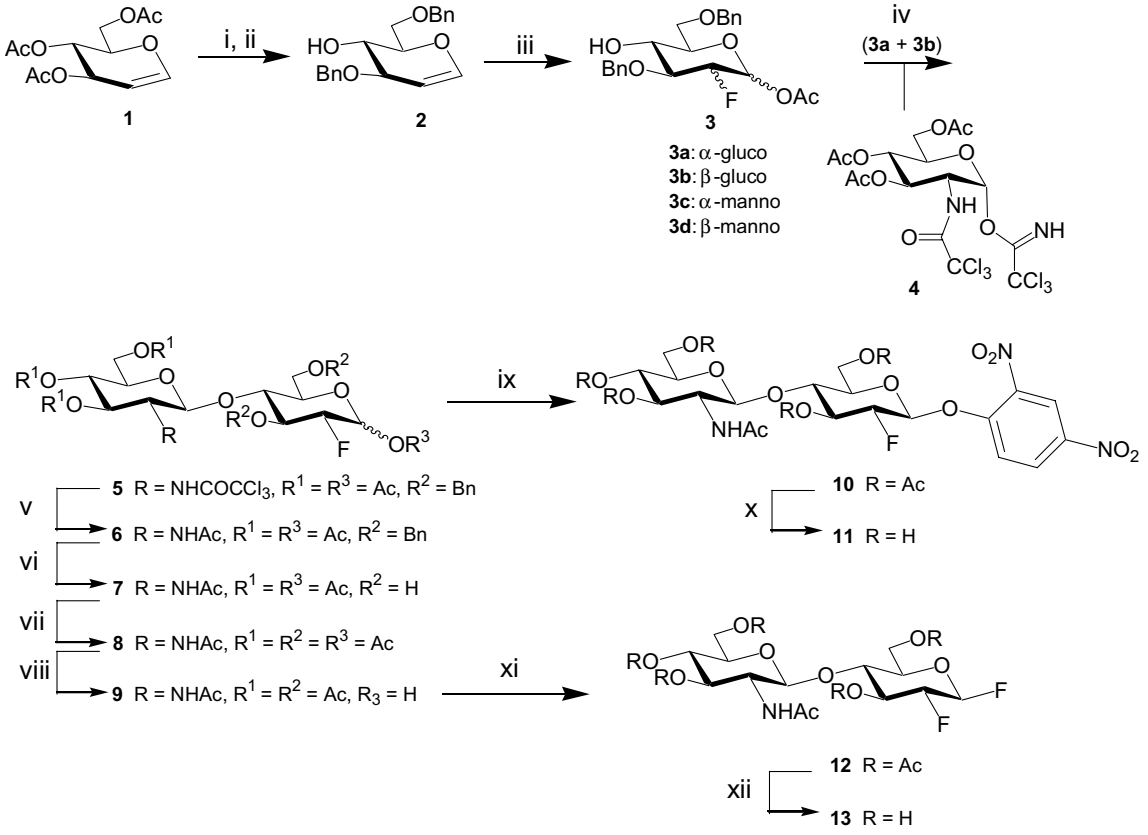
(LacZ,[8] ebg[a], ebg[b], and ebg[ab]),[9] the β-glucosidases from *Agrobacterium* sp.,[10] *Stachyobotrys atra*, *Botrydiplodia theobromae*,[11] a β-(1,4) glycanase from *Cellulomonas fimi*,[12] β-xylosidases from *Thermoanaerobacterium saccharolyticum*,[13] and *Geobacillus stearothermophilus*[14] and a β-mannosidase from *Cellulomonas fimi*.[15] In each of these cases the intermediate has been revealed as a covalent species and indeed, there is no retaining glycosidase for which the intermediate has been clearly demonstrated to be a non-covalent ion pair. Unfortunately, α-deuterium kinetic isotope effects have been unable to clarify this point with HEWL as no substrate for this enzyme has been found for which the rate-determining step is deglycosylation. Thus, in the absence of a suitable substrate for KIE experiments, we aimed to synthesize the first mechanism-based inhibitors of HEWL that function by accumulation of a stabilized intermediate.

The 2-deoxy-2-fluoro glycosyl fluorides and corresponding dinitrophenyl glycosides have proven very effective at labeling the catalytic nucleophiles of many β-retaining glycosidases. These inhibitors function by forming fairly stable covalent glycosyl-enzyme intermediates. The highly electron-withdrawing fluorine at the 2-position inductively destabilizes the oxocarbenium ion-like transition states, through which both steps of the double displacement mechanism proceed, thereby slowing both steps of the reaction. Inclusion of a very good leaving group at the anomeric center, however, accelerates the first step making the intermediate kinetically accessible. Both 2,4-dinitrophenolate and fluoride have been shown to be excellent leaving groups for use in this strategy.[16,17] Early studies with synthetic substrates revealed that HEWL was capable of cleaving disaccharide substrates and that it also had a marked preference for a fluoride-leaving group over a DNP leaving group.[18] Additionally, other studies have shown that the 2-acetamido group of the sugar in the −1 subsite is not essential for catalysis by HEWL and can be replaced by a hydroxyl group or a hydrogen.[19] Substitution of the 2-acetamido group with fluorine, which is only slightly larger than hydrogen, should therefore be tolerated by HEWL. Consequently, we opted to synthesize 2,4-dinitrophenyl 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-2-deoxy-2-fluoro-β-D-glucopyranoside (**11**) and 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-2-deoxy-2-fluoro-β-D-glucopyranosyl fluoride (**13**) as possible inactivators of HEWL. Here we report the synthesis of these compounds and the detailed kinetic analysis of their mode of action. Indeed, one of these probes, 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-2-deoxy-2-fluoro-β-D-glucopyranosyl fluoride, has recently permitted the observation of a covalent intermediate on HEWL (Chart 1) and revealed the slight structural changes in the enzyme that allow such a complex to form.[2]

2. Results and discussion

2.1. Synthesis

Preparation of the target 2-deoxy-2-fluorodisaccharides (**11** and **13**) was accomplished using a linear synthetic



Scheme 1. Synthetic route for the preparation of the target compounds NAG-2FGDNP (**11**) and NAG-2FGF (**13**). Reagents and conditions: (i) (a) NaOMe, MeOH, (b) H^+, MeOH; (ii) (a) $(Bu_3Sn)_2O$, C_6H_6, Δ, (b) TBAB, BnBr, C_6H_6, Δ; (iii) Selectfluor™, AcOH, $MeNO_2$, 25%; (iv) TMSOTf, $(CH_2Cl)_2$, 4 Å sieves, Et_3N, 0 °C, 80%; (v) Bu_3SnH, AIBN, C_6H_6, Δ, 81%; (vi) H_2, Pd–C, MeOH/EtOAc/H_2O; (vii) Ac_2O, py, 84% over two steps ; (viii) H_2NNH_3OAc, DMF, 94%; (ix) FDNB, DABCO, DMF, 55%; (x) HCl, MeOH, 26%; (xi) DAST, DCM, THF, −30 °C, 67%; (xii) (a) NaOMe, MeOH, (b) H^+, MeOH, 90%.

strategy (Scheme 1). The known, selectively benzylated, glycal **2** was prepared according to literature procedures.[20] Electrophilic fluorination of glycals has emerged as a reliable method for the preparation of 2-fluorosaccharides.[21–24] Studies have clearly demonstrated that the addition of Selectfluor™ across the glycal double bond occurs *syn* to yield 2-deoxy-2-fluoro-*N*-glycosyl intermediates.[22–24] These intermediates can be intercepted in the same pot by the addition of a wide range of nucleophiles.[21–24] We were pleased to find that addition of acetic acid during the electrophilic fluorination of glycal (**2**) using Selectfluor™ afforded the 2-deoxy-2-fluoro-D-glucopyranoses (**3a**,**b**) and -mannopyranoses (**3c**,**d**) in a ratio of 45 (**3a**+**3b**) to 55 (**3c**+**3d**) as a mixture of anomers (*gluco*, 2:3, **3a/3b**; *manno* 9:1, **3c/3d**). Purification of a mixture of the glucopyranose acceptors (**3a** and **3b**) was readily accomplished by flash column chromatography on silica. This strategy enabled us to rapidly generate the selectively protected 2-deoxy-2-fluoro-D-glucopyranoses (**3a**,**b**) bearing a free hydroxyl at the 4-position that could be used as an acceptor in the subsequent glycosylation without further manipulation. The generation of these 1-*O*-acetyl derivatives rather than interception of the *N*-glycosyl intermediate to generate the 2,4-dinitrophenyl glycoside permitted divergence of the synthetic strategy toward compounds **11** and **13** at the penultimate step. We opted to install the fluorine atom early on during the synthetic plan rather than fluorinate a disaccharide glycal in order to avoid potential problems in the separation of diastereomers later on in the synthesis. The choice of donor for the preparation of the target molecule required some consideration as it would be necessary to effect the conversion of the 2-amino protecting group into the desired acetamido functionality in the presence of the anomeric acetate of the acceptors (**3a** and **3b**). Thus, the known 2-deoxy-2-trichloroacetamide donor, **4** which is accessible[25] in four steps from commercial materials, was chosen. This trichloroacetimidate has been demonstrated to be an efficient donor for glycosylations and to also undergo facile conversion of the 2-trichloroacetamido group to an acetamide in the presence of other sensitive protecting groups. Coupling of the donor **4** to a mixture of acceptors **3a** and **3b** afforded the disaccharide **5** in good yield. Only one anomeric product was obtained from this glycosylation reaction and on the basis of

the large coupling constant (≈8.5 Hz) observed between H-1′ and H-2′ in all subsequent products, this product was deemed to be the β-anomer. This very high anomeric selectivity is consistent with previous results using this donor.[25] Subsequent reduction of the trichloroacetamido group proceeded smoothly to give the desired 2′-acetamido disaccharide **6**. Hydrogenolysis of the benzyl protecting groups of the 3,6-di-*O*-benzyl protected disaccharide **6** on a moderate scale was complicated by the presence of small amounts of residual alkyl tin from the preceding reaction, which poisoned the palladium catalyst. Rigorous purification of the disaccharide **6** was required before the removal of the benzyl groups could be effected using Pd–C in an atmosphere of hydrogen to provide diol **7**. Acetylation of the crude diol **7** to give the peracetylated disaccharide, **8** followed by selective cleavage of the anomeric acetate with hydrazine acetate yielded the hemiacetal **9** as a 1:2 mixture of β to α anomers as could be determined from both the ^{19}F and ^{1}H NMR spectra. The hydroxyl protons could be observed in the ^{1}H NMR spectrum of this anomeric mixture, with the resonance from the β anomer observed at 6.38 ppm and that of the α anomer observed at 6.22 ppm. The ratio of the intensity of these signals was consistent with that observed for the ratio measured in the ^{19}F NMR. The position of these resonances far downfield and with reasonably large coupling constants (6.6 Hz and 4.7 Hz for the β and α anomers, respectively) is consistent with the significant deshielding of these protons as seen for the hemiacetal of a 2-fluorosaccharide.[26] Reaction of disaccharide **9** with 2,4-dinitrofluorobenzene in DMF[27] gave the protected target molecule **10**. Global deprotection of glycoside **10** using HCl in methanol afforded the target 2-deoxy-2-fluoroglycoside **11**. Fluorination of the hemiacetal **9** using DAST yielded predominantly the desired β-glycosyl fluoride **12** with good stereoselectivity (β:α; 9:1). The anomeric configuration of **10** was supported by the observation of a relatively large H-1 to H-2 coupling (6.3 Hz) in the ^{1}H NMR spectrum of **10** as well as the *O*-deacetylated product **11** (7.6 Hz). The observation of a small H-1 to *F* coupling (3.7 Hz for compound **10** and 3.0 Hz for compound **11**) are also consistent with assignment of both **10** and **11** as β-configured glycosides. Indeed, the chemical shifts and coupling constants measured are in reasonable agreement with those observed by Dax and coworkers for 2,4-dinitrophenyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-fluoro-β-D-glucopyranoside.[23] Removal of the acetyl protecting groups from **12** using sodium methoxide in dry methanol provided the target compound **13**. Assignment of the anomeric configuration of compounds **12** and **13** as β-glycosyl fluorides is supported by the small coupling constant between H-1 and F-2 observed in the ^{1}H and ^{19}F NMR spectra (3.4 Hz for compound **12** and 3.3 Hz for compound **13**) and the relatively large H-1 to H-2 coupling that is observed in compounds **12** (5.6 Hz) and **13** (6.9 Hz). The magnitude of the F1 to F2 coupling (≈16 Hz) also supports assignment of compounds **12** and **13** as 2-fluoro β-glycosyl fluorides. Furthermore, the use of these compounds as substrates for HEWL in the studies described below and in earlier studies[2] published by us provide compelling evidence for the anomeric configuration of compounds **11** and **13**.

2.2. Enzymology

On incubation of **11** with HEWL (0.1 mM) and monitoring of the solution at 400 nm using a UV/Vis spectrophotometer, no reaction could be observed under any conditions. Thus, disappointingly, compound **11** is neither a substrate nor an inactivator of the enzyme. Upon incubation of **13** with HEWL, however, a steady-state release of fluoride ion was observed using a fluoride-selective electrode, indicating that this compound is cleaved by the enzyme. It is interesting to note that although the leaving group p*K*a values in each case are quite similar and many glycosidases cleave equivalent substrates with similar catalytic efficiency, HEWL discriminates between them. Ballardie and coworkers had earlier observed this behavior when studying the HEWL-catalyzed hydrolysis of β-chitobiosyl fluoride and 2,4-dinitrophenyl β-chitobioside.[18] They found that the second-order rate constant for the cleavage of the fluoride was 30-fold greater than that for the 2,4-dinitrophenyl compound and attributed it to the greater symmetry of the fluoride ion leaving group. Regardless of the physical basis of this discrimination for the aglycon moiety, the energy barrier for hydrolysis of **11** by HEWL may simply be too high for the reaction to be observed under the conditions of this study.

Kinetic analysis of the cleavage of **13** revealed that each molecule of HEWL was able to catalyze the turnover of more than one molecule of **13**. This demonstration of catalytic competence, in conjunction with the observation of linear progress curves, clearly shows that **13** is acted upon by HEWL as a substrate and not as an inactivator. A plot of the rate observed versus concentration of the substrate (Fig. 1) revealed that the apparent dissociation constant of **13** was too high to allow an accurate determination of a K_m value, which must be significantly greater than 5 mM. Unfortunately, as saturation kinetics could not be observed, it is impossible to estimate the importance of the 2-acetamido group for the binding of chitobiose and **13** to HEWL. Previous studies, however, have shown that the 2-acetamido substituent is not critical for HEWL catalyzed hydrolysis of synthetic substrates.[19] Indeed, incubation of HEWL with aryl chitobioside substrate analogues, wherein the 2-acetamido group had been replaced with either a hydroxyl or hydrogen, resulted in production of only the disaccharide, along with the liberated unnatural

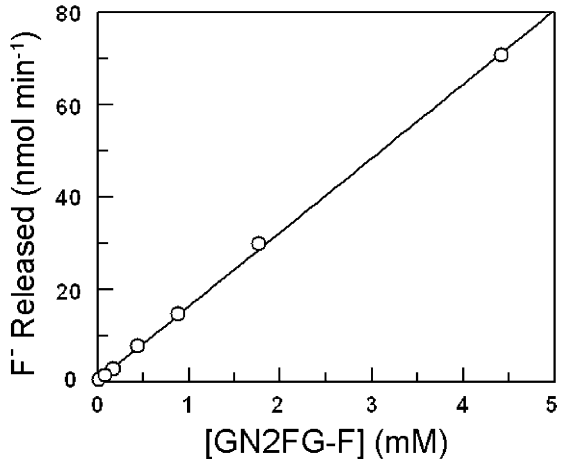


Figure 1. Michaelis–Menten plot showing the rate of fluoride release versus the concentration of NAG-2FGF in the presence of 101 μM HEWL at 25 °C in 150 mM NaOAc buffer, pH 5.00.

aglycone. Conversely, the HEWL catalyzed hydrolysis of *p*NP-chitobioside itself was found to generate a mixture of products arising from cleavage of the internal glycosidic bond in addition to the liberation of the phenol group. Replacement of the 2-acetamido group in chitobiose-based substrates with a smaller moiety thus eliminates the undesirable cleavage of the internal glycosidic bond, facilitating interpretation of the kinetic results.[19]

The apparently high K_m value and the steady-state kinetic studies showing more than one turnover of compound **13** provide some evidence that the first step of the mechanism (glycosylation) is at least partially rate determining with this substrate and indeed, this expectation has been confirmed.[2] Despite the absence of saturation kinetics, a second-order rate constant could readily be extracted from the data ($k_{cat}/K_m = 9.6\ min^{-1}\,M^{-1}$). Comparison of this value with that estimated for the hydrolysis of β-chitobiosyl fluoride by HEWL ($k_{cat}/K_m = 1200\ min^{-1}\,M^{-1}$)[18] revealed a decrease in the second-order rate constant of 126-fold. Such a decrease in the glycosylation step upon substitution of a fluorine group for the 2-acetamido group of β-chitobiosyl fluoride is small, yet is consistent with decreases found in previous reports with other glycosidases where the 2-hydroxyl of an activated substrate was replaced with fluorine. Indeed, for *Agrobacterium* sp. β-glucosidase, which is known to proceed through a covalent glycosyl enzyme intermediate, a 335-fold rate reduction was observed on substitution of the 2-hydroxyl group with fluorine.[10,17] For some other retaining β-glycosidases, much more dramatic rate reductions have been observed ranging from 1.2×10^4 to 1.0×10^6-fold.[12,28,29] That the rate reduction is so small provides further credence to a covalent glycosyl enzyme intermediate for HEWL. For a discrete oxocarbenium ion intermediate, one would reasonably expect a more significant destabilizing effect manifested as a more dramatic decrease in the second-order rate constant.

2.3. Conclusion

This paper describes in detail the synthesis and testing of two compounds designed as mechanism-based probes of HEWL. Kinetic studies using these compounds revealed that, under the conditions of this study, compound **11** was untouched by the enzyme while **13** was acted upon as a slow substrate for which the rates of glycosyl enzyme intermediate formation and breakdown were approximately equal. These results indicate that; (i) any attempts to devise 2-fluoro mechanism-based inactivators of HEWL should incorporate a fluoride-leaving group and: (ii) another method of preferentially slowing the second (deglycosylation) step over the first (glycosylation) step must be incorporated into the inhibitor design to generate a useful inhibition. Indeed stoicheometric accumulation of an intermediate with this reagent has been seen with a mutant of HEWL in which the acid/base catalyst is removed.[2]

3. Experimental

3.1. General methods

All buffer chemicals and other reagents were obtained from Sigma/Aldrich Chemical Co. unless otherwise noted. Synthetic reactions were monitored by TLC using E. Merck Kieselgel 60 F_{254} aluminum-backed sheets. Compounds were detected by charring with 10% ammonium molybdate in 2 M H_2SO_4 and heating. Flash chromatography at a positive pressure was performed with E. Merck Kieselgel 60 (230–400 mesh) using the specified eluants. ^1H NMR spectra were recorded on a Bruker WH-400 spectrometer at 400 MHz (chemical shifts quoted relative to $CDCl_3$, CD_3OD or $(CD_3)_2CO$ where appropriate). ^{19}F NMR spectra were recorded on a Bruker AC-200 at 188 MHz or a Bruker AV-300 at 282 MHz and are proton-coupled with CF_3CO_2H as a reference. ^{13}C NMR spectra were recorded on a Bruker WH-400 spectrometer at 100 MHz or a Bruker AV-300 at 75 MHz (chemical shifts quoted relative to $CDCl_3$ or to CD_3OD).

3.2. 1-*O*-Acetyl-3,6-di-*O*-benzyl-2-deoxy-2-fluoro-α-D-glucopyranose (3a) and 1-*O*-acetyl-3,6-di-*O*-benzyl-2-deoxy-2-fluoro-β-D-glucopyranose (3b)

To a soln of glycal **2** (3.60 g, 8.1 mmol) in a mixture of dry $MeNO_2$ and AcOH (5:1, 300 mL) Selectfluor™ (7.17 g, 20.3 mmol) was added portionwise over 2 h. The reaction mixture was then stirred for 16 h at room

temperature after which it was refluxed for 30 min. After cooling the reaction mixture to room temperature, the solvent was removed under diminished pressure to yield a gum that was dissolved in CH_2Cl_2 (200 mL). This CH_2Cl_2 soln was washed with water (100 mL), saturated $NaHCO_3$ (2×100 mL), water (100 mL), and brine (100 mL). The organic layer was then dried over $MgSO_4$, filtered, and concentrated under diminished pressure to a pale yellow oil. Gradient flash silica column chromatography of the residue (1:5 to 3:17, EtOAc–hexanes) yielded the desired products **3a** and **3b** as a mixture in a 3:2 ratio (1.10 g, 2.7 mmol, 25%). Further careful chromatography (3:17 EtOAc–petroleum ether (35–60 °C) to 1:5 EtOAc–petroleum ether (35–60 °C)) yielded pure samples of each anomer as gums. 1-*O*-Acetyl 3,6-di-*O*-benzyl-2-deoxy-2-fluoro-α-D-glucopyranose (**3a**); ^{19}F NMR (188 MHz, $CDCl_3$, CF_3CO_2H reference): δ −124.30 (1F, dd, $J_{F2,2}$ 49.7 Hz, F-2, $J_{F2,3}$ 12.0 Hz); ^1H NMR (400 MHz, $CDCl_3$) δ: 7.38–7.27 (10H, m, Ar × 10), 6.34 (1H, d, $J_{1,2}$ 3.9 Hz, H-1), 4.91 and 4.70 (AB quartet, 2H, J_{gem} 11.4 Hz, PhC**H**$_2$), 4.60 (1H, ddd, $J_{2,3}$ 9.2 Hz, H-2), 4.58 and 4.51 (AB quartet, 2H, J_{gem} 12.1 Hz, PhC**H**$_2$), 3.60–3.95 (5H, m, H-3, H-4, H-5, H-6, and H-6$_a$), 2.59 (1H, d, $J_{OH,4}$ 1.9 Hz, O**H**), 2.14 (3H, s, OAc); ^{13}C NMR (100 MHz, $CDCl_3$): δ 168.97 (s, CO), 138.09, 137.70, 128.56, 128.44, 128.00, 127.96, and 127.72 (s, Ph), 89.72 (d, $J_{2,F2}$ 191.35 Hz, C-2), 89.26 (d, $J_{1,F2}$ 22.97 Hz, C-1), 79.68 (d, $J_{3,F2}$ 16.37 Hz, C-3), 74.80 (d, $J_{OCH2Ph(C-3),\ F2}$ 2.26 Hz, O**C**H$_2$Ph(C-3)), 73.77, 72.21, 70.00 (d, $J_{4,F2}$ 8.70 Hz, C-4), 69.17, 20.90 (s, CH_3); Anal. Calcd for $C_{22}H_{25}FO_6$: C, 65.34; H, 6.23. Found: C, 65.07; H, 6.23.

3.3. 1-*O*-Acetyl-3,6-di-*O*-benzyl-2-deoxy-2-fluoro-β-D-glucopyranose (3b)

^{19}F NMR (188 MHz, $CDCl_3$, CF_3CO_2H reference): δ −122.70 (1F, dd, $J_{F2,2}$ 51.2 Hz, $J_{F2,3}$ 13.7 Hz, $J_{F2,1}$ 3.4 Hz); ^1H NMR (400 MHz, $CDCl_3$): δ 7.37–7.27 (10H, m, Ar ×10), 5.70 (1H, dd, $J_{1,2}$ 8.0, H-1), 4.92 and 4.90 (AB quartet, 2H, J_{gem} 11.5 Hz, PhC**H**$_2$), 4.58 and 4.51 (AB quartet, 2H, J_{gem} 12.1 Hz, PhC**H**$_2$), 4.41 (1H, ddd, $J_{2,3}$ 8.4 Hz, H-2), 3.78–3.50 (5H, m, H-3, H-4, H-5, H-6, and H-6$_a$), 2.58 (1H, d, $J_{OH,4}$ 2.3 Hz, O**H**), 2.13 (3H, s, OAc). ^{13}C NMR (75 MHz, $CDCl_3$): δ 169.19 (s, CO), 137.77, 137.54, 128.56, 128.44, 128.08, 127.84, and 127.77 (s, Ph), 91.60 (d, $J_{2,F2}$ 186.8 Hz, C-2), 91.52 (d, $J_{1,F2}$ 24.8 Hz, C-1), 82.17 (d, $J_{3,F2}$ 16.4 Hz, C-3), 74.86, 74.69 (d, $J_{OCH2Ph(C-3),F2}$ 2.3 Hz, O**C**H$_2$Ph(C-3)), 73.66, 70.27 (d, $J_{4,F2}$ 8.6 Hz, C-4), 68.95, 20.91 (s, CH_3); CIMS (NH_3): *m*/*z* 422 $(M+NH_4)^+$ (7.6%), 91 $(CH_2Ph)^+$ (100%); HRCIMS: $(M+NH_4)^+$: Calculated, 242.19788; Found, 242.19784. Also obtained from the reaction mixture was a mixture of the α and β anomers of 1-*O*-acetyl-3,6-di-*O*-benzyl-2-deoxy-2-fluoro-D-mannopyranose (**3c/d**) in a 9:1 ratio (1.3 g, 3.2 mmol, 29%).

3.4. 3,4,6-Tri-*O*-acetyl-2-deoxy-2-trichloroacetamido-β-D-glucopyranosyl-(1→4)-1-*O*-acetyl-3,6-di-*O*-benzyl-2-deoxy-2-fluoro-α/β-D-glucopyranose (5)

To acceptor **3** (654 mg, 1.62 mmol) was added the donor **4** (1.06 g, 1.78 mmol, 1.1 equiv) along with activated 4 Å sieves. After the addition of 1,2-dichloroethane (10 mL) the reaction mixture was stirred for 1 h at room temperature under an atmosphere of dry argon. The reaction mixture was then cooled to 0 °C and trimethylsilyltriflate (60 μL) was added. After 2 h, analysis of the mixture by TLC revealed that the acceptor had been completely consumed and Et_3N was added (0.5 mL). The reaction mixture was diluted with CH_2Cl_2 (5 mL) and filtered. The filtrate was then concentrated to a pale yellow residue under diminished pressure. Careful flash chromatography of this residue on a silica column with gradient elution (4:1 petroleum ether (35–60 °C)–EtOAc to 3:1 petroleum ether (35–60 °C)–EtOAc) yielded the desired product **5** as a mixture of anomers (1.009 g, 1.21 mmol, 75%). ^{19}F NMR (188 MHz, $CDCl_3$, CF_3CO_2H reference): δ −122.26 (1F, dd, $J_{F2,H2}$ 51.9 Hz, $J_{F2,H3}$ 15.4 Hz, $J_{F2,H1}$ 3.3 Hz F-2, β-anomer), −124.30 (1F, dd, $J_{F2,H2}$ 47.8 Hz, $J_{F2,H3}$ 12.2 Hz, α-anomer); Selected ^1H NMR data (400 MHz, $CDCl_3$): δ 7.50–7.21 (10H, m), 6.33 (0.65H, d, $J_{1,2}$ 4.0 Hz, H-1, α anomer), 6.16 (0.65H, d, $J_{NH,2}$ 9.2 Hz, NH, α-anomer), 6.15 (0.35H, d, $J_{NH,2}$ 9.0 Hz, NH, β anomer), 5.62 (0.35H, dd, $J_{1,2}$ 8.1 Hz, H-1, β-anomer), 5.02–4.67 (5H, m), 4.52 (0.65H, ddd, $J_{2,3}$ 9.2 Hz, H-2, α-anomer), 4.36–4.25 (2.35H, m), 4.17–4.11 (1H, m), 4.04–3.83 (4H, m), 3.73–3.60 (2H, m), 3.54–3.35 (2H, s); CIMS (NH_3): *m*/*z* 857 $(M(^{35}Cl^{37}Cl_2)+NH_4)^+$ (10.5%), 855 $(M(^{35}Cl_2{}^{37}Cl)+NH_4)^+$ (38.0%), 853 $(M+NH_4)^+$ (26.7%); HRCIMS: $(M+NH_4)^+$: Calculated, 853.19202; Found, 853.19537, $(M(^{35}Cl_2{}^{37}Cl)+NH_4)^+$ Calculated, 855.18909; Found, 855.18989. Anal. Calcd for $C_{36}H_{41}Cl_3FNO_{14}{\cdot}0.5H_2O$: C, 51.11; H, 5.00; N, 1.66. Found: C, 51.00; H, 4.92; N 1.90.

3.5. 2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl-(1→4)-1-*O*-acetyl-3,6-di-*O*-benzyl-2-deoxy-2-fluoro-α/β-D-glucopyranose (6)

To disaccharide **5** (642 mg, 0.76 mmol) was added a spatula tip of AIBN followed by benzene (7.5 mL) and tributyltin hydride (1.25 mL, 4.6 mmol, 6 equiv). This reaction mixture was stirred for 1 h under dry argon at room temperature and then refluxed for 2 h. After cooling the reaction to room temperature, the solvent was removed under diminished pressure to yield a white

solid. This residue was dissolved in MeCN (50 mL) and washed with hexanes (3 × 50 mL). The MeCN phase was concentrated under diminished pressure to yield **6** as a white crystalline solid. Flash chromatography of this residue on silica with an initial step (1:1 EtOAc–petroleum ether (35–60 °C)) followed by gradient elution (4:1 EtOAc–petroleum ether (35–60 °C) to 3:1 EtOAc–petroleum ether (35–60 °C)) yielded the desired product **6** as fine white crystals (451 mg, 0.61 mmol, 81%). An anomerically pure analytical sample was obtained during the column chromatography: 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl-(1→4)-1-*O*-acetyl-3,6-di-*O*-benzyl-2-deoxy-2-fluoro-α-D-glucopyranose (**6a**). ^{19}F NMR (188 MHz, $CDCl_3$, CF_3CO_2H reference): δ −121.90 (1F, dd, $J_{F2,H2}$ 51.0 Hz, $J_{F2,H3}$ 16.0 Hz, $J_{F2,H1}$ 4.0 Hz F-2, β-anomer), −124.11 (1F, dd, $J_{F2,H2}$ 47.3 Hz, $J_{F2,H1}$ 11.9 Hz, α-anomer); ^{1}H NMR (400 MHz, $CdCl_3$): δ 6.31 (1H, d, $J_{1,2}$ 4.0 Hz, H-1), 4.97 (1H, dd, $J_{4',3'}$ 9.6 Hz, $J_{4',5'}$ 9.6 Hz, H-4′) 4.91 and 4.71 (AB quartet, 2H, J_{gem} 11.7 Hz, PhC**H**$_2$), 4.83 (1H, dd, $J_{3',2'}$ 10.0 Hz, H-3′), 4.81 and 4.34 (AB quartet, 2H, J_{gem} 12.1 Hz, PhC**H**$_2$), 4.59 (1H, d, $J_{NH,2'}$ 9.3 Hz, NH), 4.53 (1H, ddd, $J_{2,3}$ 8.8 Hz, H-2), 4.41 (1H, d, $J_{1',2'}$ 8.5 Hz, H-1′), 4.09 (1H, dd, J_{gem} 12.3 Hz, $J_{6',5'}$ 4.4 Hz, H-6′), 3.96–3.88 (3H, m, H-2′, H-3, H-4), 3.85 (1H, dd, $J_{6a',5'}$ 2.4 Hz, H-6$_{a'}$), 3.74–3.70 (1H, m, H-5), 3.61 (1H, dd, J_{gem} 11.0, $J_{6,5}$ 2.4 Hz, H-6), 3.49 (1H, dd, $J_{6a,5}$ 2.0 Hz, H-6$_a$), 3.42 (1H, ddd, H-5′); 2.13, 1.99, 1.97, 1.96, 1.92, 1.69 (s each, 3H each, 5 × COC**H**$_3$); ^{13}C NMR (75 MHz, $CDCl_3$): δ 182.98, 170.75, 170.60, 169.83, 169.30, and 169.15 (s, CO), 138.67, 137.50, 129.09, 129.00, 128.13, 127.37, 127.33, and 127.03 (s, Ph), 100.93 (s, C-1′), 88.90 (d, $J_{1,F2}$ 22.8 Hz, C-1), 88.84 (d, $J_{2,F2}$ 191.2 Hz, C-2), 78.40 (d, $J_{3,F2}$ 17.2 Hz, C-3), 76.10 (d, $J_{4,F2}$ 8.4 Hz, C-4), 74.67 (s), 73.88 (s), 72.75 (s), 71.85 (s), 71.42 (s), 68.22 (s), 66.68 (s), 61.74 (s, C-6′), 54.14 (s, C-2′), 23.12 (CH_3), 20.98 (s, CH3), 20.61 (s, CH3); CIMS$^+$ (NH_3): *m*/*z* 751 $(M+NH_4)^+$ (0.46%); 734 $(M+H)^+$ (14.1%); 674 (25.6%); HRCIMS: $(M+NH_4)^+$: Calculated, 751.30896; Found, 751.30957; $(M+H)^+$: Calculated, 734.27968; Found, 734.28241.

3.6. 2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl-(1→4)-1,3,6-tri-*O*-acetyl-2-deoxy-2-fluoro-α/β-D-glucopyranose (8)

The disaccharide **6** (335 mg, 0.46 mmol) was dissolved in a mixture of 6:2:1 MeOH–EtOAc–water (9 mL). Pd–C (340 mg) was added and the reaction mixture was stirred under an atmosphere of hydrogen for 4 d at which time the reaction was judged to be complete by ^{19}F NMR. The catalyst was removed by suction filtration through Celite and glass fiber and the solvent removed under diminished pressure to yield crude **7** as a clear residue. To this gum was added pyridine (6 mL) and Ac_2O (2 mL). After stirring this mixture for 16 h, the solvent was evaporated under diminished pressure to give a tan powder. The desired product was then purified by flash chromatography on a column of silica with gradient elution (1:4 petroleum ether (35–60 °C)–EtOAc to 1:6 petroleum ether (35–60 °C)–EtOAc) yielding the desired product **8** as an off white powder (240 mg, 0.38 mmol, 84%). A small amount of the pure α anomer **8a** was obtained during the chromatography; ^{19}F NMR (188 MHz, $CDCl_3$, CF_3CO_2H reference): δ −124.22 (1F, dd, $J_{F2,H2}$ 48.5 Hz, $J_{F2,H3}$ 15.4 Hz, F-2); ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.33 (1H, d, $J_{1,2}$ 3.9 Hz, H-1), 5.85 (1H, d, $J_{NH,2'}$ 8.8 Hz, NH), 5.50 (1H, ddd, $J_{3,F2}$ 12.0 Hz, $J_{3,2}$ 9.5 Hz, $J_{3,4}$ 9.5 Hz, H-3), 5.24 (1H, dd, $J_{2',3'}$ 10.4 Hz, $J_{3',4'}$ 9.3 Hz, H-3′), 5.02 (1H, dd, $J_{4',5'}$ 9.5 Hz, H-4′), 4.63 (1H, d, $J_{1',2'}$ 3 Hz, H-1′), 4.52 (1H, ddd, H-2), 4.40–4.22 (3H, m, H-6, H-6$_a$, H-6′), 4.04–3.96 (2H, m, H-5, H-6′$_a$), 3.76 (1H, ddd, H-2′), 3.68 (1H, dd, H-4), 3.63 (1H, ddd, $J_{5',6'}$ 4.3 Hz, $J_{5',6a'}$ 2.4 Hz, H-5′), 2.17 (3H, s, NAc), 2.14, 2.10, 2.08, 2.06, 1.99, 1.98, and 1.91 (3H each, s each, 5 × $COCH_3$); ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.97, 170.64, 170.50, 170.40, 169.72, and 169.34, 168.84 (s, CO), 100.77 (s, C-1′), 88.17 (d, $J_{1,F2}$ 22.27 Hz, C-1), 86.35 (d, $J_{2,F2}$ 196.46 Hz, C-2), 75.33 (d, $J_{4,F2}$ 7.26 Hz, C-4), 72.14, 71.83, 70.54 (s, C-2′, C-3′, C-4′), 70.03, (d, $J_{3,F2}$ 19.07 Hz, C-3), 68.05 (s), 61.71, 61.44 (s each, C-6, C-6′), 54.97 (s, C-2′), 23.16 (s, NHCO**C**H$_3$), 20.91, 20.71, 20.66, 20.59 (s, 5 × CO**C**H$_3$); Selected NMR data for the β anomer; ^{19}F NMR (188 MHz, $CDCl_3$, CF_3CO_2H reference): δ −125.91 (1F, dd, $J_{F2,H1}$ 12.2 Hz, $J_{F2,H2}$ 49.0 Hz, F-2); CIMS (NH_3): *m*/*z* 655 $(M+NH_4)^+$ (2.0%); 638 $(M+H)^+$ (63.0%); HRCIMS $(M+H)^+$: Calculated, 638.20978; Found, 638.20966; Anal. Calcd for $C_{26}H_{36}FNO_{16}$: C, 48.98; H, 5.69; N, 2.20. Found: C, 48.85; H, 5.69; N 2.14.

3.7. 2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl-(1→4)-3,6-di-*O*-acetyl-2-deoxy-2-fluoro-α/β-D-glucopyranose (9)

To a soln of **8** (230 mg, 0.36 mmol) in DMF (3 mL) was added hydrazine acetate (45 mg, 0.49 mmol, 1.4 equiv) and the reaction mixture was stirred at room temperature for 2 h. After this time, water (20 mL) was added and a white precipitate formed immediately. This mixture was extracted with CH_2Cl_2 (2 × 25 mL) and the organic layer was then washed with water (25 mL), twice with satd $NaHCO_3$ (2 × 25 mL), and once with brine (25 mL). The organic layer was then dried with $MgSO_4$, filtered, and the solvent removed under diminished pressure to yield **9** as a mixture of anomers (1:2, β to α) appearing as a faintly yellow solid (202 mg, 0.34 mmol, 94%). ^{19}F NMR (188 MHz, $(CD_3)_2CO$, CF_3CO_2H reference): δ −117.96 (1F, ddd, $J_{F2,H2}$ 51.2 Hz, $J_{F2,H3}$

14.5 Hz, $J_{F2,H1}$ 2.3, F-2 (β anomer)), −118.94 (1F, dd, $J_{F2,H2}$ 50.5 Hz, $J_{F2,H3}$ 12.3 Hz, F-2 (α anomer)); ^{1}H NMR (400 MHz, $(CD_3)_2CO$): 7.14 (d, $J_{NH,2'}$ 8.4, Hz, NH), 7.13 (d, $J_{NH,2'}$ 8.2, Hz, NH), 6.38 (d, $J_{OH,1}$ 6.6, Hz, **OH** β-anomer), 6.22 (d, $J_{OH,1}$ 4.7, Hz, **OH** α-anomer), 5.54 (ddd, *J* 11.9 Hz, *J* 9.5 Hz, *J* 9.5 Hz), 5.40–5.25 (m), 5.0–4.92 (m), 4.52–4.32 (m), 4.15–4.00 (m), 4.88–3.63 (m), 2.104, 2.096 (s, NHAc), 2.062, 2.057, 2.051, 2.046, 2.033, 1.957, 1.920, 1.916, 1.830, 1.824 (s, OAc); CIMS (NH_3): *m/z* 596 $(M+H)^+$ (7.4%), 536 (14.5%), 330 (100%); HRCIMS: $(M+H)^+$: Calculated, 596.19910; Found, 596.20166.

3.8. 2,4-Dinitrophenyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl-(1→4)-3,6-di-*O*-acetyl-2-deoxy-2-fluoro-β-D-glucopyranoside (10)

To a soln of hemiacetal **9** (93 mg, 0.16 mmol) in DMF (1.8 mL) under argon was added 2,4-dinitrofluorobenzene (65 mg, 0.34 mmol, 2.2 equiv) and DABCO (82 mg, 0.73 mmol, 4.7 equiv). The dark orange soln was stirred at room temperature for 3.5 h after which CH_2Cl_2 (60 mL) was added. This soln was washed with water (1 × 50 mL), satd $NaHCO_3$ (2 × 50 mL), and brine (2 × 50 mL). The organic layer was dried over $MgSO_4$, filtered, and concentrated under diminished pressure to a yellow powder. This residue was then partially purified by step gradient flash column chromatography on silica (1:3 petroleum ether–EtOAc to 1:4 MeOH–EtOAc) to yield a pale yellow solid containing a mixture of anomers. Crystallization of this solid from EtOAc–EtOH–hexane yielded the pure β-anomer **10** as fine white crystals (67 mg, 0.088 mmol, 55%); mp 258–259 °C (dec); ^{19}F NMR (188 MHz, $CDCl_3$, CF_3CO_2H reference): δ −120.85 (1F, ddd, $J_{F,H2}$ 49.8 Hz, $J_{F,H3}$ 16.6 Hz, $J_{F,H1}$ 3.7 Hz); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.70 (1H, d, $J_{AR3,AR6}$ 8.4 Hz, H_{AR3}), 8.41 (1H, dd, $J_{AR6,AR5}$ 9.7 Hz, H_{AR6}), 7.37 (1H, d, H_{AR5}), 5.76 (1H, d, $J_{NH,H2'}$ 8.5 Hz, NH), 5.43 (1H, d, $J_{1,2}$ 6.3 Hz, H-1), 5.38 (1H, ddd, $J_{3,2}$ 7.9 Hz, $J_{3,4}$ 7.9 Hz, H-3), 5.28 (1H, dd, $J_{H3',H2'}$ 10.3 Hz, $J_{H3',H4'}$ 9.4 Hz, H-3′), 5.03 (1H, dd, $J_{H4',H5'}$ 9.6 Hz, H-4′), 4.74 (1H, d, $J_{H1',2'}$ 8.2 Hz, H-1′), 4.63 (1H, ddd, H-2), 4.42 (1H, dd, $J_{H6,\ H6a}$ 12.2 Hz, $J_{H6,\ H5}$ 1.7 Hz, H-6), 4.35 (1H, dd, $J_{H6',H6a'}$ 12.4 Hz, $J_{H6',H5'}$ 4.3 Hz, H-6′), 4.19 (1H, dd, $J_{H6a,\ H5}$ 4.5 Hz, H-6_a), 4.06 (1H, dd, $J_{H6a',H5'}$ 2.2 Hz, H-$6'_a$), 3.95–3.86 (2H, m, H-5, H-4), 3.74–3.64 (2H, m, H-2′, H-5′), 2.13, 2.08, 2.02, 2.00, 1.99, 1.91 (3H each, s each, 5 × $COCH_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 182.98, 170.65, 170.51, 170.45, 170.40, and 169.65, 169.36 (s, CO), 153.28, 142.18, 140.23, 128.57, 121.55, 117.70 (s, Ph) 100.50 (s, C-1′), 97.87 (d, $J_{1,F1}$ 26.56 Hz, C-1), 88.65 (d, $J_{2,F1}$ 191.04 Hz, C-2), 74.86 (d, $J_{4,F1}$ 5.75 Hz, C-4), 73.54, 72.14, 71.98 (s, C-2′, C-3′, C-4′), 72.06, (d, $J_{3,F2}$ 16.33 Hz, C-3), 68.23 (s), 61.80, 61.58 (s, C6, C6′), 55.37 (s, C-2′), 23.19 (s, $NHCOCH_3$), 20.71, 20.67, 20.63, 20.58 (s, 5 × $COCH_3$); Anal. Calcd for $C_{28}H_{34}FN_3O_{18}$; C, 46.74; H, 4.76; N, 5.84. Found: C, 47.00; H, 4.74; N 5.53.

3.9. 2,4-Dinitrophenyl 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-2-deoxy-2-fluoro-β-D-glucopyranoside (11)

To a cool (0 °C) soln of the per-*O*-acetylated glycoside **10** (22 mg, 0.029 mmol) in dry MeOH (1 mL) was added AcCl (0.2 mL) to yield a 2.3 M soln of methanolic HCl. This mixture was then maintained at 0 °C for 20 h at which point the solvent was evaporated under diminished pressure to yield a pale yellow crystalline residue. Recrystallization of this material from MeOH yielded pale yellow needles of **11** (4.2 mg, 0.008 mmol, 26%). ^{19}F NMR (376 MHz, CD_3OD, CF_3CO_2H reference): δ −124.60 (1F, ddd, $J_{F,H2}$ 51.2 Hz, $J_{F,H3}$ 16.5 Hz, $J_{F,H1}$ 3.0 Hz); ^{1}H NMR (400 MHz, CD_3OD): δ 8.72 (1H, d, $J_{AR3,AR5}$ 2.8 Hz, H_{AR3}), 8.46 (1H, dd, $J_{AR5,AR6}$ 9.3 Hz, H_{AR6}), 7.61 (1H, d, H_{AR6}), 5.60 (1H, d, $J_{H1,\ H2}$ 7.6 Hz, H-1), 4.51 (1H, d, $J_{H1',H2'}$ 8.5 Hz, H-1′), 4.35 (1H, ddd, $J_{H2,H3}$ 8.7 Hz, H-2), 3.95 (1H, ddd, $J_{H3,\ H4}$ 7.9 Hz, H-3), 3.91 (1H, dd, $J_{H6,\ H6a}$ 11.6 Hz, $J_{H6,\ H5}$ 2.0 Hz, H-6), 3.84 (1H, d, $J_{H6',H6a'}$ 11.6 Hz, H-6′), 3.73–3.62 (5H, m, H-6a, H-3′, H-4′, H-5′, H-6a′), 3.44 (1H, dd, $J_{H4,\ H5}$ 10.3 Hz, H-4), 3.35 (1H, ddd, $J_{H5,\ H6a}$ 6.5 Hz, H-5), 1.99 (3H, s, CH_3); ^{13}C NMR (100 MHz, CD_3OD): δ 173.78 (s, CO), 154.90, 143.13, 141.15, 129.74, 122.16, 118.90, 103.16, 99.19 (d, $J_{1,F1}$ 23.78 Hz, C-1), 92.37 (d, $J_{2,F1}$ 189.20 Hz, C-2), 80.11 (d, $J_{4,F1}$ 7.63 Hz, C-4), 78.15 (s, C-5′), 77.06 (s, C-5), 75.77 (s, C-3′), 74.58, (d, $J_{3,F1}$ 17.89 Hz, C-3), 72.05 (s, C-4′), 62.61, 61.18 (s, C6, C6′), 57.33 (s, C-2′), 23.04 (s, $NHCOCH_3$); HRESIMS: $(M+NH_3)^+$: Calculated, 574.1297; Found, 574.1308; $(M+H^+)$: Calculated, 552.1477; Found 552.1470.

3.10. 2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl-(1→4)-3,6-di-*O*-acetyl-2-deoxy-2-fluoro-β-D-glucopyranosyl fluoride (12)

To a cool (−30 °C) soln of the hemiacetal **9**, under an atmosphere of argon, (90 mg, 0.15 mmol) in a mixture of CH_2Cl_2 (3 mL) and THF (10 mL) was added DAST (0.5 mL, 0.38 mmol). The reaction mixture was warmed to 20 °C and stirred for 30 min. The reaction was judged to be complete by TLC analysis of the mixture and was then cooled to −30 °C at which time 100 μL of MeOH were added. This mixture was then warmed to 20 °C and stirred for another 3 h. The solvent was then evaporated under diminished pressure to yield a dark yellow gum, which was dissolved in CH_2Cl_2 (30 mL), washed with satd $NaHCO_3$ (30 mL) and brine (30 mL), and dried over $MgSO_4$. After concentration under dimin-

ished pressure, the resulting gum was purified by flash column silica chromatography (1:4 petroleum ether (35–60 °C)–EtOAc) to provide a white crystalline solid. Recrystallization of this material from a mixture of EtOAc and EtOH provided fine white needles of **12** (60 mg, 0.1 mmol, 67%); mp 258–259 °C; ^{19}F NMR (282 MHz, $CDCl_3$, CF_3CO_2H reference): δ −138.65 (ddd, $J_{F1,H1}$ 52.8 Hz, $J_{F1,F2}$ 17.0 Hz, F-1), −199.25 (dddd, $J_{F2,H2}$ 49.6 Hz, $J_{F2,H3}$ 16.0 Hz, $J_{F2,H1}$ 3.4 Hz, F-2); ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.72 (1H, d, $J_{NH,H2'}$ 8.4 Hz, NH), 5.40 (1H, ddd, $J_{1,2}$ 5.6 Hz, Hz, H-1), 5.29 (2H, m, H-3, H-3′), 5.01 (1H, dd, $J_{H4',H3'}$ $J_{H4',H5'}$ 9.6 Hz, H-4′), 4.74 (1H, dd, $J_{H1',H2'}$ 8.3 Hz, H-1′), 4.46 (1H, m, H-2), 4.42 (1H, d, $J_{H6,H6a}$ 12.2 Hz, H-6), 4.34 (1H, dd, $J_{H6',H6a'}$ 12.4 Hz, $J_{H6',H5'}$ 4.6 Hz, H-6′), 4.21 (1H, dd, $J_{H6,H5}$ 2.8 Hz, H-6_a), 4.02 (1H, dd, $J_{H6a',H5'}$ 2.2 Hz, H-$6'_a$), 3.83 (2H, m, H-5, H-4), 3.71–3.63 (2H, m, H-2′, H-5′), 2.12, 2.09, 2.07, 2.00, 1.99, 1.91 (3H each, s each, 5 × $COCH_3$); ^{13}C NMR (100 MHz, $CdCl_3$): δ 170.80, 170.59, 170.41, 170.32, 169.52, and 169.36 (s, CO), 105.63 (dd, $J_{1,F1}$ 218.7 Hz, $J_{1,F2}$ 28.6 Hz, C-1), 100.40 (s, C-1′), 88.96 (dd, $J_{2,F2}$ 189.6 Hz, $J_{2,F1}$ 30.2 Hz, C-2), 74.79 (d, $J_{5,F1}$ 6.0 Hz, C-5), 72.90 (s, C-4), 71.95 (s, 2 × C), 71.66 (dd, $J_{3,F2}$ 13.8 Hz, $J_{3,F1}$ 8.1 Hz, C-3), 68.22, 61.80, 61.66, 55.36 (s), 23.19 (s, NHCO**C**H_3), 20.87, 20.64, 20.59, 20.57 (s, 5 × CO**C**H_3); Anal. Calcd for $C_{24}H_{33}F_2NO_{14}$; C, 48.24; H, 5.57; N, 2.34. Found: C, 48.45; H, 5.59; N 2.39.

3.11. 2-Acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-2-deoxy-2-fluoro-β-D-glucopyranosyl fluoride (13)

To a soln of **12** (24 mg, 0.04 mmol) in 2 mL of dry MeOH was added 50 mL of a 1 M soln of NaOMe in dry MeOH. After 30 min, the reaction was judged complete and Amberlite IR-120 (H^+) cation exchange resin was added until the mixture was neutral. The soln was then filtered and the solvent evaporated under diminished pressure to yield a pale yellow gum. Preparative thin layer chromatography of this gum (7:2:1 EtOAc–MeOH–water) yielded the desired product as a transparent gum (14 mg, 0.036 mmol, 90%). ^{19}F NMR (282 MHz, CD_3OD, CF_3CO_2H reference): δ −145.59 (1F, ddd, $J_{F1,H1}$ 53.5 Hz, $J_{F1,F2}$ 14.1 Hz, $J_{F1,H2}$ 13.3 Hz, F-1), −203.64 (1F, dddd, $J_{F2,H2}$ 51.4 Hz, $J_{F2,H3}$ = 17.0 Hz, $J_{F2,H1}$ = 3.3 Hz, F-2); ^{1}H NMR (400 MHz, CD_3OD) δ: 5.35 (1H, d, $J_{1,2}$ 6.9 Hz, H-1), 4.49 (1H, d, $J_{1',2'}$ 8.5 Hz, H-1′), 4.15 (1H, dddd, $J_{2,3}$ 8.5 Hz, H-2), 3.90 (1H, dd, $J_{H6,H6a}$ 11.6 Hz, $J_{H6,H5}$ 2.2 Hz, H-6), 3.85 (1H, ddd, $J_{H3,F2}$ 17.0 Hz, $J_{3,4}$ 8.5 Hz, H-3), 3.80 (1H, dd, $J_{H6',H6a'}$ 12.2 Hz, $J_{H6',H5'}$ 2.0 Hz, H-6′), 3.67 (1H, dd, $J_{H4',H5'}$ 10.3 Hz, $J_{H4',H3'}$ 8.6 Hz, H-4′), 3.64–3.60 (3H, m, H-6a, H-6a′, H-3′), 3.50 (1H, ddd, $J_{H5,H4}$ 10.3 Hz, $J_{H5,H6a}$ 4.4 Hz, H-5), 3.43 (1H, dd, H-4), 3.33 (1H, ddd, $J_{H5,H6a'}$ 6.5 Hz, H-5′), 3.30–3.26 (1H, m, H-2′), 1.8 (3H, s, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.76 (s, CO), (Note: C-1 was not observed above noise but is expected to be appear as a dd at approximately 105 ppm), 103.11 (s, C-1′), 83.2 (dd, $J_{2,F2}$ 190.2 Hz, $J_{2,F1}$ 28.4 Hz, C-2), 79.98 (d, $J_{5,F1}$ 6.8 Hz, C-5), 78.12, 76.48 (d, $J_{4,F2}$ 9.0 Hz, C-4), 75.76, 74.02 (dd, $J_{3,F2}$ 14.6 Hz, $J_{3,F1}$ 8.1 Hz, C-3), 72.06, 62.61, 61.15, 57.34 (s, C-2′), 23.01 (s, NHCO**C**H_3); CIMS (NH_3): *m/z* 388 $(M+H)^+$ (100%); 368 $(M-F)^+$ (12.3%); HRCIMS $(M+H)^+$: Calculated, 388.14191; Found, 388.14280.

Enzyme kinetics: HEWL was obtained from Sigma Chemical Co (Lot # 89F8276). All kinetic studies were performed at 25 °C in 150 mM NaOAc buffer, pH 5.00. Attempts to observe the liberation of DNP from **11** (5 mM) in the presence of HEWL (101 μM) were made by monitoring the reaction at 400 nm by means of a UNICAM 8700 UV–vis spectrophotometer equipped with a circulating water bath. Reaction rates for the hydrolysis of **13** by HEWL were determined by monitoring the release of fluoride using a 9606BN Ionplus Orion fluoride ion electrode interfaced to a pH/ion selective meter (Fischer Scientific). Reactions were initiated by the addition of an aliquot (50 μL) of a stock soln of enzyme (12.7 mg/mL) to give a final concentration of 101 μM HEWL in a final vol of 440 μL. Reaction cells were pre-equilibrated in a water bath to 25 °C. Calculation of the second-order rate constant for substrate **13** was carried out by directly fitting the initial rate data to a linear equation using GraFit version 3.0.[30]

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