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Ortho- and *para*-(Difluoromethyl)aryl-β-D-glucosides: A New Class of Enzyme-Activated Irreversible Inhibitors of β-Glucosidases

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New *ortho*- and *para*-(difluoromethyl)aryl-β-D-glucosides were stereoselectively prepared in three steps from 1-bromo-2,3,4,6-tetraacetyl glucose, using an appropriate *ortho*- or *para*-hydroxybenzaldehyde derivative. The fluorine atoms were introduced by reacting the so-formed *ortho*- or *para*-*O*-glucosyl benzaldehyde derivatives with DAST. Title compounds are potent time-dependent irreversible inhibitors of almond β-glucosidase. The inactivation is explained by the enzyme-catalyzed hydrolysis of the glucosidic linkage, releasing an *ortho*- or *para*-(difluoromethyl)phenol. The *ortho*- and *para*-(difluoromethyl)phenols are assumed to rapidly form fluorinated quinone methides which alkylate a nucleophilic residue of the enzyme active site.

INTRODUCTION

Glycosidase inhibitors have been used successfully as tools to study the intrinsic mechanism of a variety of catalyzing glycoside hydrolysis (*1*). Furthermore, they have permitted the elucidation of the successive steps involved in glycoprotein processing and an evaluation of the physiological importance of these steps (*2*). Ultimately, they have a high potential as therapeutic agents in various diseases including diabetes (*3*), obesity, (*3*) and viral infections (*4*).

In the design of glycosidase inhibitors, chemists have to choose between two main classes of compounds, high-affinity reversible inhibitors, and enzyme-activated irreversible inhibitors. Lead compounds of the first class include the naturally occurring inhibitors acarbose (*3*), valiolamine (*5*), nojirimycin (*3*), castanospermine (*6*), deoxymannojirimycin (*2, 7*), and swainsonine (*2, 8*). They are all sugar analogues with a nitrogen replacing one of the anomeric oxygen atoms. The high affinity for their target enzymes is explained by favorable electrostatic interactions between a carboxylate of the enzyme active site and the protonated amino group of the inhibitor (*1, 3, 6–8*). On the other hand, only a few examples of enzyme-activated irreversible inhibitors of glycosidases have been reported to date. They include conduritol epoxide **1** (*9*), glucosylmethyltriazene **2** (*10*), the aziridine triol derivative of piperidine **3** (*11*), and, more recently, conduritol aziri-

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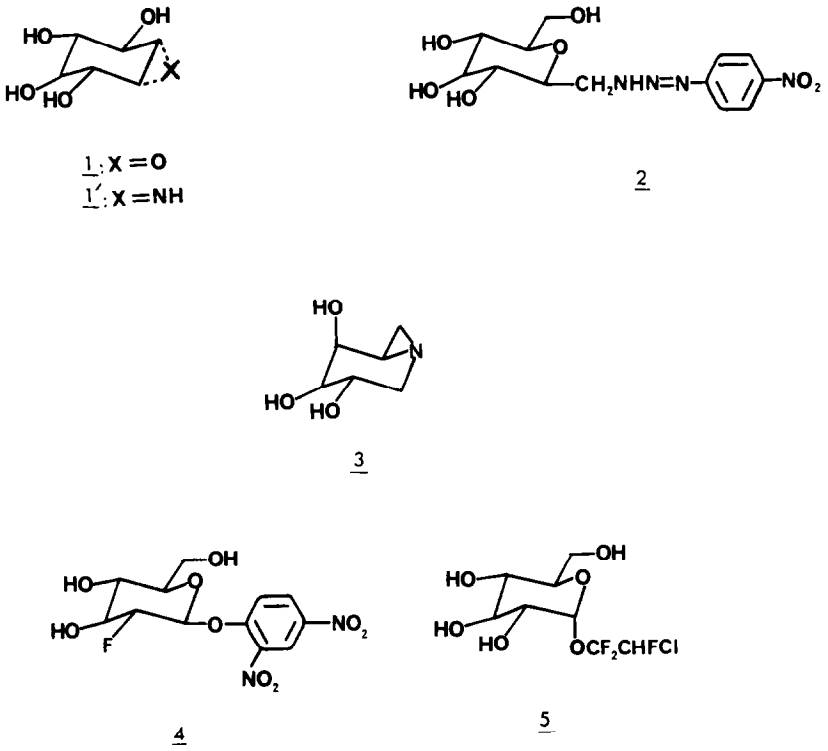
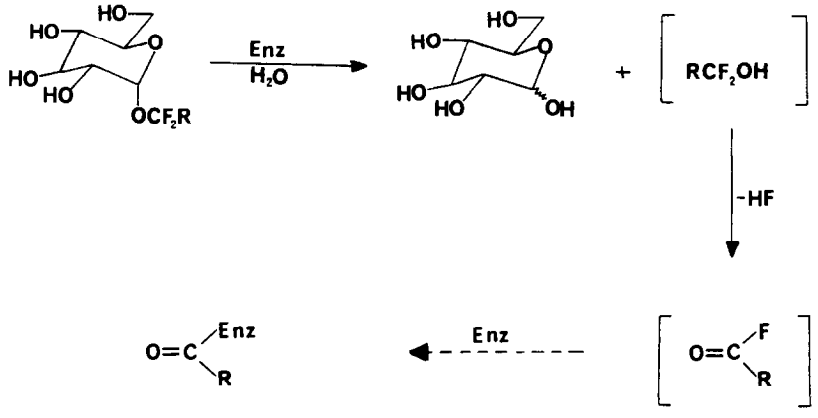


FIG. 1. Structure of some mechanism-based inhibitors of glucosidases.

dine **1'** (*12*) (see Fig. 1). These compounds are activated upon enzyme-catalyzed protonation into highly reactive species which are believed to form a covalent bond with some nucleophilic residue of the enzyme. However, they have a high intrinsic chemical reactivity since their functional groups (epoxide, triazene, or aziridine) are activated upon simple protonation, and, therefore, insufficient specificity could limit their use as drugs.

Inhibitors activated only after or during enzymatic cleavage of the glycosidic bond should be more selective. Such an approach has recently been illustrated by two different examples of enzyme-activated irreversible inhibitors of glycosidases in which one or two fluorine atoms have been introduced in the glycosyl or in the aglycone portion of the inhibitor, respectively. The first example is 2,4-dinitrophenyl-2-deoxy-2-fluoro-β-D-glucopyranoside **(4)** (*13*). This compound, a time-dependent inhibitor of *Alcaligenes faecalis* β-glucosidase, is believed to be cleaved before inactivating the enzyme by the formation of a covalent glycosyl–enzyme complex. The other example is the 1',1'-difluoroalkyl glucoside **5** (Fig. 1). Recently, we reported (*14*) that **5** produced a time-dependent irreversible inhibition of yeast α-glucosidase. We showed that **5** was cleaved before inactivating the enzyme and we suggested the formation of a covalent aglycone–enzyme bond (*14*). Our results concerning the inhibition of yeast α-glucosidase by **5** were in agreement with a



SCHEME 1

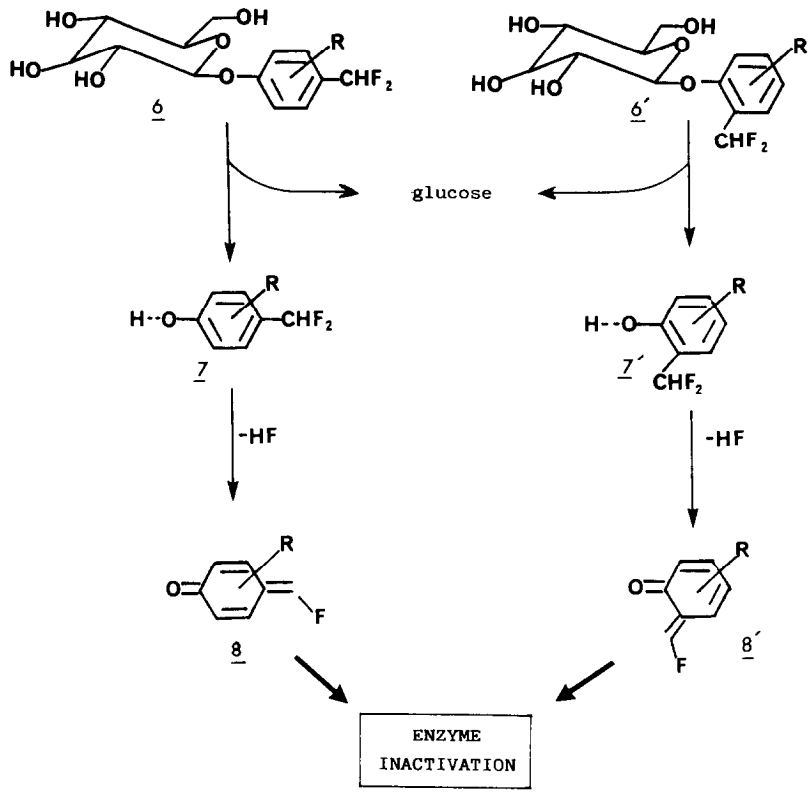
novel process of enzyme-activated inhibition (Scheme 1) based on the reactivity of the leaving group, i.e., the unstable alcohol RCF_2OH, released during the glucosidase-catalyzed hydrolysis of a stable unreactive glucoside. Furthering this rationale, any glucoside, which would be hydrolyzed to an alcohol rapidly decomposing to an electrophilic species, could be a potential enzyme-activated irreversible inhibitor of glucosidases.

In the present work, we considered the possibility of generating an unstable phenol through the glucosidase-catalyzed hydrolysis of a stable arylglucoside. A similar approach was previously reported for the inhibition of proteases or esterases by *ortho-* or *para-*(chloromethyl)phenylesters or amides (*15, 16*). However, benzylchlorides may behave as alkylating agents and produce nonspecific inhibition of enzymes. On the other hand, benzylfluorides, and more particularly benzyldifluorides, are stable and nonalkylating compounds. Therefore, we designated the *ortho-* or *para-*(difluoromethyl)aryl-β-glucosides **6** or **6'** (Scheme 2) as potential inhibitors of β-glucosidase. The products of the β-glucosidase-catalyzed hydrolysis of **6** and **6'**, i.e., the *ortho-* and *para-*difluoromethylphenols **7** and **7'**, are expected (*17*) to rapidly loose HF and to be transformed into the reactive fluorinated quinone methides **8** or **8'**, respectively. These intermediates are reactive electrophilic species which may form a covalent adduct with a nucleophilic residue of the enzyme-active site. Here, we describe the synthesis of **6** and **6'** and we show that these compounds represent a new class of stable time-dependent inhibitors of almond-β-glucosidase, on the basis of the principle discussed above.

RESULTS AND DISCUSSION

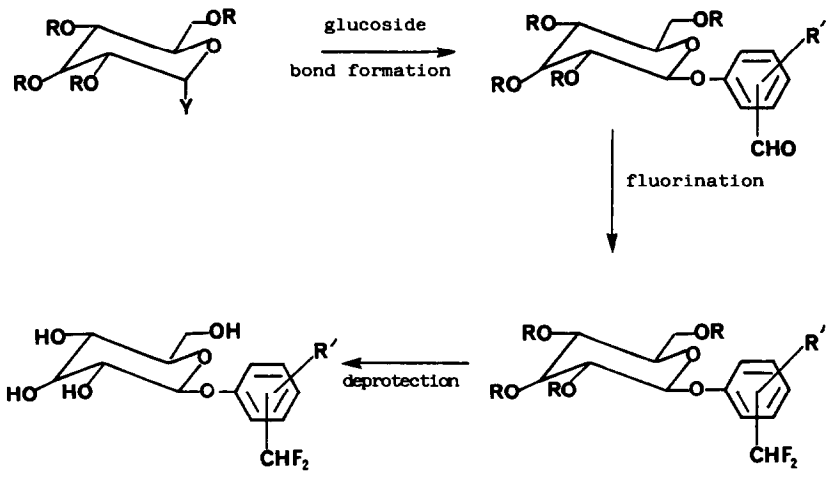
A. Chemistry

Aryl-β-glucosides were usually prepared by condensing the corresponding phenols with an appropriate glucose derivative activated in an anomeric position and having other hydroxyl groups protected. Since the *ortho-* and *para-*(difluorometh-



SCHEME 2

yl)phenols **7** and **7'** were expected to rapidly loose HF, these compounds were not used as such for glucosidic bond formation. Instead, the preparation of **6** or **6'** required the stereoselective β-glucosidic bond formation to be achieved prior to the introduction of the fluorine atoms. Thus, we have performed the preparation of the target derivatives **6** or **6'** according to the general Scheme 3. In order to stereoselectively prepare the β-anomer by taking advantage of the participating effect in 2-position, 1-bromo-2,3,4,6-tetraacetyl-α-D-glucose was used as the starting material. Accordingly, we found that the phase-transfer-catalyzed β-glucosylation procedure (*18, 19*) could be applied to the coupling of *ortho-* or *para*-hydroxybenzaldehyde derivatives with 1-bromo-2,3,4,6-tetracetyl-α-D-glucose (Scheme 4, Step 1). The yields of the glucosidic bond formation step were found to be somewhat modest if the published experimental procedure (*19*) was strictly applied. However, when the reaction was performed in a more diluted aqueous sodium hydroxyde solution and for a longer period of time, the products **9** were isolated in better yields as indicated by values between brackets in Table 1. Moreover, this method allowed the preparation of the β-anomer with an anomeric selectivity higher than 98% since no α-anomer could be detected by ^{1}H NMR analysis. This was in contrast with the well-known silver salt procedure (*20*), which requires

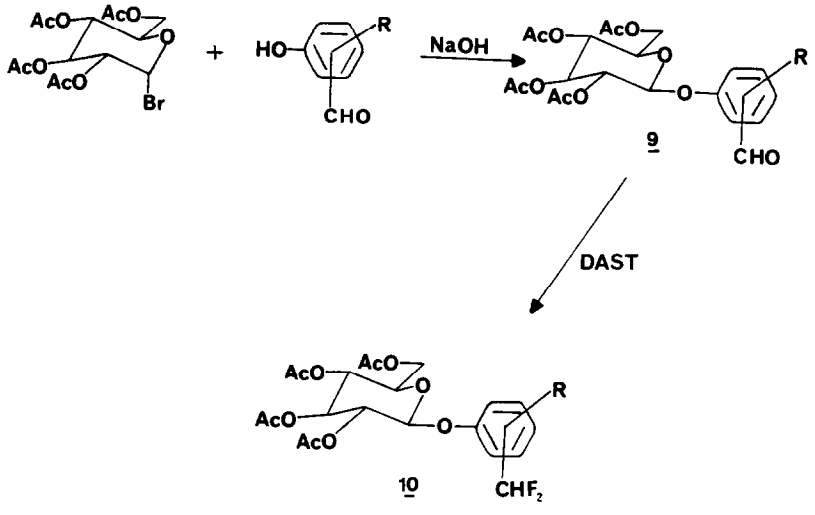


SCHEME 3

expensive reagents and gives the expected glucoside in a similar yield but with less control of the β-selectivity (as we observed in one of our cases).

The second step was successfully achieved by treating the tetra-*O*-acetyl-β-D-(glucopyranosyloxy)benzaldehyde) derivatives **9** with excess of DAST (*21*) in dichloromethane (Scheme 4, Step 2). The difluorobenzylglucosides **10** were isolated in a pure form after flash chromatography purification on silica gel.

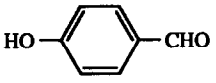
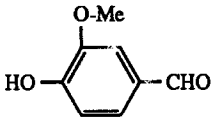
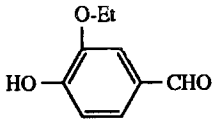
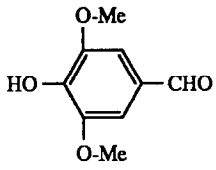
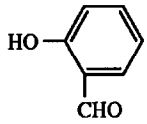
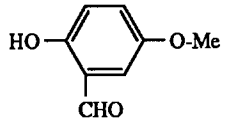
The final products **6** and **6′** were easily obtained from the intermediates **10** after



SCHEME 4

TABLE 1

Experimental Details of Scheme 4

Entry	ArOH	Yield of 9 (%)	Yield of 10 (%)
a		30	85
b		46 (58)	80
c		42	82
d		40	85
e		33	85
f		33 (58)	70

reaction with catalytic amounts of sodium methoxide in methanol at 20°C. This reaction was almost quantitative and the products were obtained in a pure analytical form (Table 2) after crystallization from methanol (yields between 60 and 90%). The β-stereochemistry of all final products was demonstrated by ^{1}H NMR analysis: in all cases, the anomeric proton appeared as a doublet with J_{H1-H2} = 7.3 to 7.6 Hz, and no α-anomeric proton could be detected. ^{19}F NMR Analysis of the final products showed an interesting different pattern between *para*- and *ortho*-difluorobenzylglucosides: in the cases of the products **6a–6d,** ^{19}F NMR showed a doublet due to the coupling of the two stereoidentical fluorine atoms with the benzylic hydrogen (J_{H-F} = 56 to 58 Hz). In the cases of the final products **6e** and **6f,** NMR spectra showed that the two fluorine atoms were different (ABX pattern): each of them was coupled to the benzylic hydrogen (J_{H-F} = 55 Hz) and they were coupled together (J_{F-f} = 310 Hz) (Table 3).

TABLE 2

Physicochemical Data and Elemental Analysis

Compound	uv		Elemental analysis		mp (°C)
	λ (nm)	(ε)	Calculated	Found	
	223	(10,520)	C: 50.90	50.42	
6a	268	(833)			160–161
	274	(716)	H: 5.27	5.29	
	206	(18,833)	C: 50.00	49.50	
6b	230	(8,494)			194–195
	277	(2,610)	H: 5.39	5.57	
	205	(24,035)	C: 51.43	51.42	
6c	229	(8,536)			193–194
	277	(2,512)	H: 5.75	5.88	
	209	(33,961)	C: 49.18	48.48	
6d	234	(6,407)			230–233
	274	(1,450)	H: 5.50	5.52	
	212	(7,220)	C: 50.98	51.02	
6e	271	(1,646)			173–174
	276	(1,454)	H: 5.27	5.31	
	202	(15,770)	C: 50.00	49.32	
6f	226	(9,297)			195–197
	292	(3,079)	H: 5.39	5.33	
	205	(18,438)	C: 52.83	52.12	
11	229	(8,002)			138
	278	(2,482)	H: 6.02	6.17	

For comparison purpose, we have also prepared the *ortho*-(methoxy)-*para*-(monofluoromethyl)aryl-β-D-glucoside **11** in three steps from 3-methoxy-4-(2,3,4,6-tetraacetyl-β-D-glucopyranosyloxy)benzaldehyde **9b** according to Scheme 5. As depicted, the reduction of the aldehyde **9b** into the alcohol **12** was better achieved by using sodium cyanoborohydride under acidic conditions (85% yield). It is worth noting that, when the reaction is carried out with sodium borohydride ($NaBH_4$), a different reduction product (45% yield) was obtained in which acetylation of the benzyl alcohol was observed. This result may be due to an acetyl tranfer from one of the glucose acetyl group to the *in situ*-produced benzyl alcoholate. This migration was completely avoided under acidic conditions using sodium cyanoborohydride. The two subsequent reactions (Scheme 5) were easily performed, the fluorination of the benzyl alcohol with DAST giving **13** in a 57% yield; deprotection with sodium methoxide in methanol gave **11** after neutralization and crystallization in methanol.

B. Biochemistry

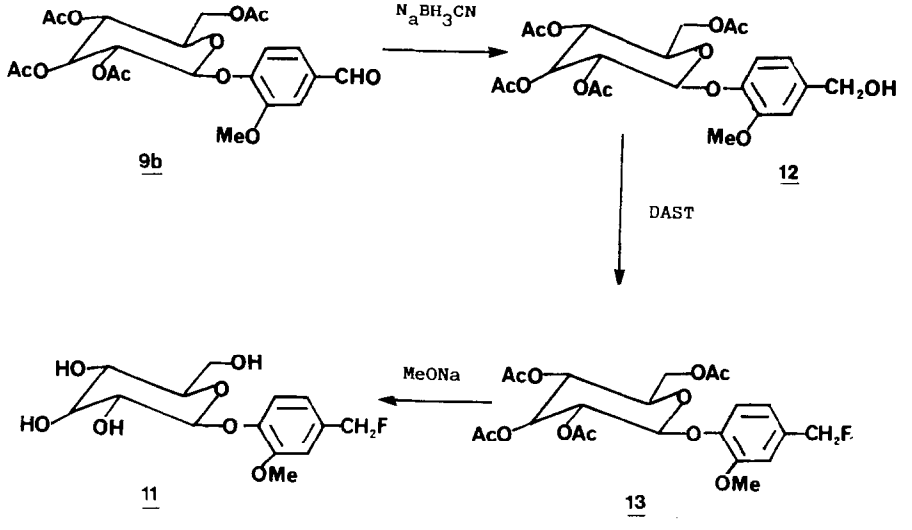
Incubation of β-glucosidase from almond with compounds **6a–6f,** or **11,** resulted in a time-dependent loss of enzyme activity which followed first-order kinetics (Fig. 2). The rate of inactivation was related to the concentration of inhibitor. By

TABLE 3

NMR Data

cpd	H_1	H_{2-6}	H_{arom}	H_{benz} + H_{OR}	F
6a	5.0; d (J_{H1-H2} = 7.6 Hz)	3.7–3.9; m (2H) 3.3–3.5; m (4H)	7.45; d (2H) 7.15; d (2H)	6.65; t (12H; J_{H-F} = 57 Hz)	55.4; d (J_{H-F} = 57 Hz)
6b	5.0; d (J_{H1-H2} = 7.4 Hz)	3.7–3.9; m (2H) 3.3–3.6; m (4H)	7.25; d (1H) 7.15; d (1H) 7.08; d (1H)	6.7; t (2H; J_{H-F} = 58 Hz) 3.85; s (3H)	55; d (J_{H-F} = 58 Hz)
6c	5.05; d (J_{H1-H2} = 7.5 Hz)	3.6–3.9; m (2H) 3.4–3.5; m (4H)	7.25; d (1H) 7.2; s (1H) 7.1; d (1H)	6.75; t (2H; J_{H-F} = 56 Hz) 4.15; q (2H) 1.45; t (3H)	55; d (J_{H-F} = 56 Hz)
6d	5.15; d (J_{H1-H2} = 7.7 Hz)	3.6–4; m (5H) 3.4; m (1H)	7.1; s (2H)	6.9; t (2H; J_{H-F} = 56 Hz) 4.08; s (6H)	56; d (J_{H-F} = 56 Hz)
6e	5.05; d (J_{H1-H2} = 7.3 Hz)	3.4–3.85; m (6H)	7.65; d (1H) 7.55; dd (1H) 7.3; d (1H) 7.25; dd (1H)	7.22; t (1H; J_{H-F} = 55 Hz)	51,25 and 47,5 ABX type J_{H-F} = 55 Hz J_{F-F} = 300 Hz
6f	4.78; d (J_{H1-H2} = 7.6 Hz)	3.6–3.8; m (2H) 3.3–3.5; m (4H)	7.25; d (1H) 7.0; m (2H)	7.15; t (1H; J_{H-F} = 55 Hz) 3.75; s (3H)	48.3 and 50.8 ABX type J_{H-F} = 55 Hz J_{F-F} = 303 Hz
11	4.95; d (J_{H1-H2} = 7.4 Hz)	3.6–3.9; m (2H) 3.3–3.6; m (4H)	7.2; d (1H) 7.05; s (1H) 6.95; d (1H)	5.3; d (2H; J_{H-F} = 48 Hz) 3.85; s (3H)	−38.05 (t, J_{F-H} = 48 Hz)

plotting the time of half-inactivation as a function of the reciprocal of the inhibitor concentration according to the method of Kitz and Wilson (*22*), a straight line was obtained (Fig. 2). This line did not pass through the origin but intercepted the positive *y* axis, demonstrating a saturation effect which involves the enzyme's active site in the inhibitory process. Kinetic constants for the time-dependent inhibition of β-glucosidase, i.e., the apparent dissociation constants (K_I) and the times of half-inactivation to infinite concentration of inhibitor ($\tau_{1/2}$) were extrapo-



SCHEME 5

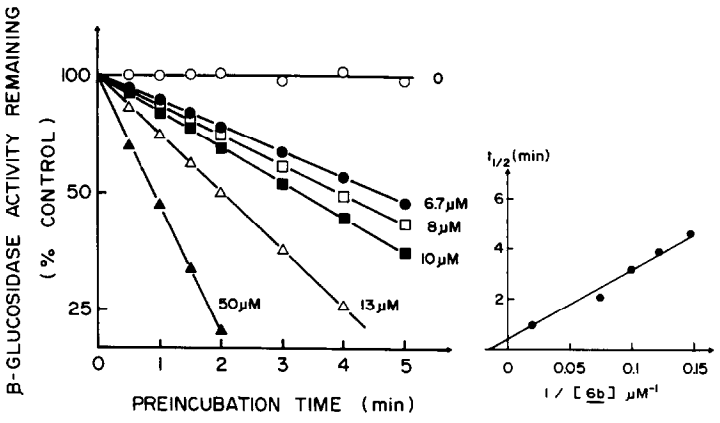


FIG. 2. Time- and concentration-dependent inhibition of almond β-glucosidase by **6b**. The enzyme was incubated at 37°C with 0.05 M sodium acetate buffer (pH 5.6) and the indicated concentrations of **6b**. At given time intervals, aliquots were withdrawn and assayed for the remaining activity. In the righthand part of the figure, the times of half-inactivation ($t_{1/2}$) are plotted against the reciprocal of the **6b** concentrations, according to Kitz and Wilson (*22*).

lated from such Kitz and Wilson replots. Values of K_I and $\tau_{1/2}$ are given for compounds **6a–6f,** and **11,** in Table 4.

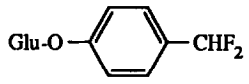
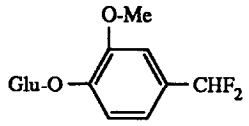
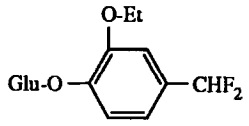
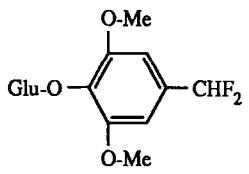
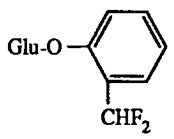
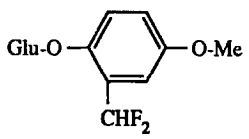
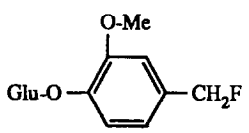
Further studies on β-glucosidase inactivation by **6b** showed protective effects of deoxynojirimycin, a competitive inhibitor of the enzyme (*23*) (Fig. 3). This confirms that the active site is involved in the inactivation process. Incubation of β-glucosidase with 100 μM **6b** for 10 min resulted in 90% inactivation. Prolonged dialysis of the inactivated enzyme for 24 h at 4°C did not regenerate enzyme activity, suggesting a covalent linkage of the inhibitor to the enzyme's active site. Addition of 5 mM dithiothreitol or β-mercaptoethanol to the preincubation medium protected the enzyme against inactivation (Fig. 3) but did not restore activity of the enzyme previously inhibited by **6b.** The protective effects of nucleophilic thiols may suggest that the species responsible for inactivation was released from the enzyme's active site before alkylating the enzyme (*24, 25*). However, such an assumption would be in contradiction with the apparent absence of a lag-time before the onset of inhibition, especially if the rate of enzyme alkylation is low. It is possible that the thiols may enter the active site and compete with active site nucleophiles for the activated electrophilic semiquinones (*25*). Alternatively, an oxidized dithiol (E ) might be necessary in the inactivation process. Qualitatively, similar results were obtained with compounds **6a–6f,** and **11.**

CONCLUSION

In conclusion, we have shown for the first time that *ortho-* and *para-*(mono- or difluoromethyl)aryl-β-D-glucosides are enzyme-activated irreversible inhibitors of almond β-glucosidase. The biochemical data reported in this paper are in agree-

TABLE 4

Time-Dependent Inhibition of β-Glucosidase from Almond

Compound	Structure	K_I (mM)	$\tau_{1/2}$ (min)
6a		1.7 ± 0.4	1.7 ± 0.3
6b		0.06 ± 0.01	0.4 ± 0.1
6c		0.10 ± 0.02	0.6 ± 0.1
6d		1.9 ± 0.2	0.5 ± 0.1
6e		3.7 ± 0.5	3.0 ± 0.2
6f		0.9 ± 0.1	1.8 ± 0.1
11		0.8 ± 0.4	0.5 ± 0.3

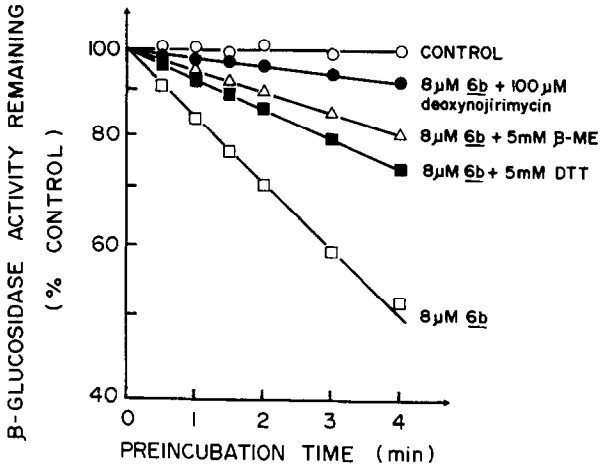


FIG. 3. Protective effects of dithiothreitol (DTT), β-mercaptoethanol (β-ME), and deoxynojirimycin on the inactivation of almond β-glucosidase by **6b**.

ment with a novel process of enzyme-activated inhibition due to the inactivation property of the leaving group released during the glucosidase-catalyzed hydrolysis. Comparison of kinetic constants, and more particularly of $\tau_{1/2}$ values, indicate that the *ortho*-difluoromethyl substituted inhibitors are not more active than the *para*-analogues (e.g., **6e** versus **6a,** Table 4) whereas *ortho*-toluylglucoside has been shown to be hydrolyzed 50-fold more rapidly than *para*-toluylglucoside (*26*). This suggests that glycosidic bond cleavage may not be the rate-limiting step in the inhibition by compounds **6.** Moreover, the comparison of kinetic constants shows that *para*-monofluoromethylphenol, and possibly the *ortho*-analogue, have the same potential as the difluoromethylphenols to form reactive quinone methides, in agreement with theoretical considerations (*17*).

Thus, it appears from this work and from our previous publication (*14*) that the use of substrates releasing reactive leaving groups may constitute a general approach to the design of irreversible inhibitors of hydrolytic enzymes.

EXPERIMENTAL

General

All reactions carried out at room temperature were at or near 20°C. Solvents were dried or distilled before use. Unless otherwise indicated, all reactions were carried out under an inert atmosphere of argon with stirring. During workup, solvents were removed with a rotary evaporator unless otherwise stated.

Proton and fluorine magnetic resonance spectra were recorded on either a varian-EM 390 NMR spectrometer (90 MHz) or a Brüker instrument (360 MHz). Chemical shifts are reported in δ units, parts per million (ppm) downfield from TMS for ^{1}H NMR, and from hexafluorobenzene for ^{19}F NMR.

Materials

All analytical TLC work was done with Kieselgel $60F_{254}$ plates (Merck, 0.2-mm layer). Chromatographic purifications were carried out with Kieselgel 60 as the solid phase (230–400 mesh), from E. Merck Laboratories. α-D-Glucose pentaacetate, 4-hydroxybenzaldehyde, vanillin, 3-ethoxy-4-hydroxybenzaldehyde, syringaldehyde, salicylaldehyde, 2-hydroxy-5-methoxybenzaldehyde, and sodium cyanoborohydride were purchased from Janssen Chimica; diethylaminosulfur trifluoride was purchased from Aldrich; *para*-nitrophenyl-β-D-glucopyranoside, deoxynojirimycin, and partially purified β-glucosidase from almonds, type I (specific activity: 51 μmol × min^{-1} (mg protein)$^{-1}$ when measured at 37°C, pH 5.6, using *para*-nitrophenyl-β-D-glucopyranoside as the substrate), were purchased from Sigma Chemical Co. All other biochemicals were of the purest grade commercially available.

Assay of β-Glucosidase

The enzyme activity was measured by monitoring the increase in absorbance at 400 nm due to hydrolysis of *para*-nitrophenyl-β-D-glucopyranoside. All assays were carried out in the presence of 0.05 M sodium acetate buffer (pH 5.6) and 0.1 mM EDTA, at 37°C.

Assay of Time-Dependent Inhibition of β-Glucosidase

For a typical experiment 160 μl of enzyme preparation containing 13 μg of protein was mixed at time zero with 40 μl of a solution of inhibitor in 0.05 M sodium acetate buffer (pH 5.6) and incubated at 37°C in a shaking water bath. At various times, 20-μl aliquots were transferred into a 980-μl assay medium and assayed for the remaining activity.

SYNTHETIC PROCEDURES

Preparation of 1-Bromo-2,3,4,6-tetraacetyl-α-D-glucose

A 275-ml aliquot of A 33% solution of hydrobromic acid in acetic acid was added to 28.1 g (72 mmol) of α-D-glucose pentaacetate at 0°C. The reaction mixture was stirred at 20°C for 3 h and extracted with chloroform (3 × 150 ml). The combined extracts were washed with cold water several times (until pH reaches 6–7), dried over sodium sulfate, filtered, and evaporated to give 29 g of a brown viscous oil which was used in the next step without further purification.

Phase-Transfer-Catalyzed β-Glucosylation Procedure

Typical example: synthesis of 3-methoxy-4-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyloxy)benzaldehyde, **9b.**

A solution of vanillin (6.4 g, 42 mmol) in dichloromethane (50 ml) was vigourously stirred at room temperature with 5% aqueous sodium hydroxyde (70 ml) and

tetrabutylammonium bromide (7 mmol, 2.26 g). To this stirred mixture was added a solution of 2,3,4,6-tetraacetyl-α-D-glucopyranosylbromide (11.5 g, 27.9 mmol) in dichloromethane (20 ml) and stirring was continued at room temperature for 40 h. The two phases were then separated. The organic layer was washed with 5% sodium hydroxyde solution (2 × 20 ml) and several times with water, dried (sodium sulfate), filtered, and evaporated *in vacuo* to give 14 g of crude product which was purified by flash chromatography on silicagel (using 7 : 3 petroleum ether : ethyl acetate) affording 7.83 g (58%) of 3-methoxy-4-(2,3,4,6-tetra-*O*-acetyl)β-D-glucopyranosyloxy)benzaldehyde, **9b.**

^{1}H NMR ($CDCl_3$): δ 9.95 (s, 1H), 7.4 (m, 2H), 7.25 (s, 1H), 5–5.5 (m, 5H), 4.1–4.2 (m, 2H), 3.75 (s, 3H), 2.05 (s, 12H).

Fluorination Procedure

Typical example: synthesis of 3-methoxy-4-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyloxy)difluoromethylbenzene **10b.**

One milliliter of diethylaminosulfur trifluoride (DAST) was slowly added to a stirred solution of 1 g (2.07 mmol) of 3-methoxy-4-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyloxy)benzaldehyde (**9b**) in 4 ml of anhydrous dichloromethane at 0°C. The reaction mixture was stirred at 20°C for 18 h, quenched slowly at 0°C with 1 ml of methanol, and evaporated *in vacuo*. The residue was directly purified by flash chromatography on silica gel (using 8 : 2 petroleum ether : ethyl acetate) giving 850 mg of product **10b** (85% yield).

^{1}H NMR ($CDCl_3$): β 6.95–7.25 (m,3H), 6.7 (t, 1H, J_{H-F} = 45 Hz), 5–5.5 (m, 5H), 4.1–4.2 (m, 2H), 3.7 (s, 3H), 2.05 (s, 12H).

Deacetylation Procedure

Typical example: synthesis of 3-methoxy-4-(β-D-glucopyranosyloxy)difluoromethylbenzene (**6b**).

1.75-mmol sample of **10b** (880 mg) was dissolved in 14 ml of methanol containing 13 mg of sodium methoxide at 20°C. After 2 h the reaction mixture was quenched with 0.2 ml of 1 N HCl, filtered, and evaporated. The final product, **6b,** is crystallized from hot methanol (95% yield, 563 mg).

*Sodium Cyanoborohydride Reduction of **9b***

A 173-mg sample (2.65 mmol) of sodium cyanoborohydride was added to a solution of **9b** (2.56 g, 5.3 mmol) and a few milligrams of bromocresol in 15 ml of THF and 11 ml of methanol. The reaction mixture turned blue and a few drops of 2 N HCl were added from time to time to maintain the yellow coloration. After 1 h, 170 mg of sodium cyanoborohydride was added, followed by drops of HCl to keep the solution yellow. After 2 h, nitrogen was bubbled through the reaction mixture, which was diluted in ethylacetate, washed with bicarbonate and brine, dried over sodium sulfate, filtered, evaporated, and purified by flash chromatogra-

phy on silicagel (using 6 : 4 petroleum ether : ethyl acetate), affording 2.2 g of pure product **13** (85% yield).

^{1}H NMR: δ 6.75–7.2 (m, 3H), 4.75–5.3 (m, 4H), 4.5 (s, 2H), 3.8–4.3 (m, 3H), 3.75 (s, 3H), 2.6 (M, 1H).

Synthesis of ***14***

DAST (0.35 ml, 2.8 mmol) was slowly added to a stirred solution of **13** (0.7 g, 1.4 mmol) in 4 ml of anhydrous dichloromethane. The reaction mixture was stirred at room temperature for 18 h, quenched at 0°C with 0.4 ml of methanol, evaporated *in vacuo,* and purified by flash chromatography on silica gel (using 8 : 2 petroleum ether : ethyl acetate) affording 0.38 g of product **14** (57% yield).

^{1}H NMR: δ 6.9–7.3 (3H, m), 5.35 (2H, d, J_{H-F} = 46.5 Hz), 5.0–5.4 (4H, m), 3.85–4.35 (3H, m), 3.75 (3H, s), 2.1 (12H, m).

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