Synthesis and evaluation of new 6-deoxy-6-fluorinated glucosides as inhibitors of yeast α-glucosidase

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Abstract

A series of 6-deoxy-6-fluorinated glucosides have been synthesized and evaluated as inhibitors of yeast α-glucosidase. These compounds have a 7- to 25-fold higher affinity than the corresponding 6-hydroxy derivatives. The most potent inhibitor in the series was the new compound 6-deoxy-6-fluoro-(3-trimethylsilyl)propyl-α-D-glucopyranoside (**3e**) prepared by condensing 2,3,4-tri-*O*-benzyl-6-deoxy-6-fluoro-α-D-glucosylchloride with 3-trimethylsilylpropanol.

Introduction

Fluorine substitution for either hydrogen or hydroxy groups in biochemically active molecules is based on the compactness and on the intense electronegativity of the fluorine atom [1, 2]. These properties, as well as the ability of fluorine to function as a hydrogen-bond acceptor [3, 4], often permit the construction of inhibitory analogues of substrates for enzymes normally acting on hydroxy compounds [1–5]. In this respect, deoxyfluorinated carbohydrates have been the subject of extensive studies during the past decades [2, 6–8].

The mechanism of action of α-glucosidases has been shown to proceed via a glucosyl oxocarbonium ion intermediate or, at least, via a transition state having substantial oxocarbonium ion character [9]. Substitution of a hydroxy group adjacent to the pyranose ring oxygen by an electronegative fluorine atom has resulted in significant destabilization of the oxocarbonium ion and, thus, in stabilization of the glucoside towards the α-glucosidase-catalyzed hydrolytic reactions [10–15]. Furthermore, it has been shown that the 6-hydroxy group is essential to permit α-D-glucosides to be the substrates of yeast α-glucosidase [16]. However, to our knowledge, 6-deoxyfluorinated α-D-glucosides have never been examined as inhibitors of the enzyme. Here, we report the inhibition of yeast α-glucosidase by a known compound [17], phenyl-6-deoxy-6-fluoro-α-D-glucopyranoside. In addition to the introduction of fluorine atom in position 6, we have also studied the modification of the aglycon part of the inhibitors. The 'aglycon site' of glucosidases is known to be built up from hydrophobic groups for a number of enzymes including

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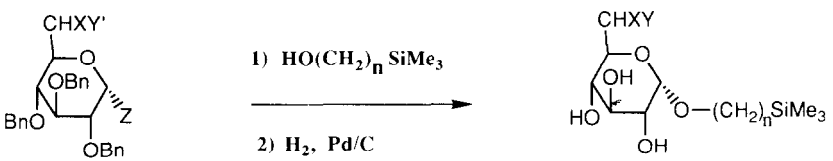
α-glucosidases [16, 18] as well as β-glucosidases [19]. In the enzyme–substrate complex, these groups contribute to the binding of substrates such as aryl- and alkyl-glucosides. In that respect, we describe in this report the synthesis and inhibitory properties of new glucosides and 6-deoxy-6-fluorinated derivatives having a trimethylsilylated carbon chain as the aglycon moiety.

Results and discussion

Phenyl-6-deoxy-6-fluoro-α-D-glucopyranoside (**1**) was prepared according to a published procedure [17].

The new [20] 1-(2-trimethylsilylethyl)- and 1-(3-trimethylsilylpropyl)-α-D-glucopyranosides (**3a**) and (**3b**) have been prepared by condensing 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl bromide [21] (**2**) (X=Br, Y=OBn, Z=H) with 2-trimethylsilylethanol and 3-trimethylsilylpropanol respectively using the halide ion-catalyzed procedure [21]. The 6-deoxy-6-fluoro derivatives were obtained using the same procedure but starting from 2,3,4-tri-*O*-benzyl-6-deoxy-6-fluoro-α-D-glucopyranosylchloride (**2**) (X=Cl, Y=F, Z=H) which was obtained (Scheme 2) in one step by reacting the known methyl-2,3,4-tri-*O*-benzyl-6-deoxy-6-fluoro-α-D-glucopyranoside [22] with boron trichloride [23, 24]. It is noteworthy that the silver salt procedure (Scheme 1, entry d) gives a lower yield of the expected product with a lower control of α-stereoselectivity when compared to the halide ion procedure as illustrated in one case (Scheme 1, entries c and d).

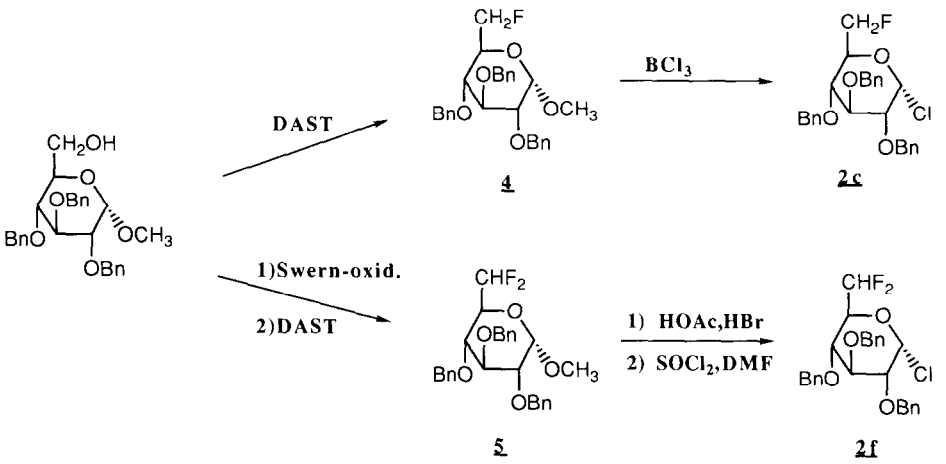
The 6-deoxy-6,6-difluoro derivative **3f** has also been prepared by the same method from 2,3,4-tri-*O*-benzyl-6-deoxy-6,6-difluoro-α-D-glucopyranosylchloride (**2f**) (X=Cl, Y=Z=F) which was obtained in four steps according to Scheme 2. Thus, the known [25] methyl-2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranoside was first oxidized into the corresponding aldehyde using



2 **3**

Entry	Z	X	Y'	Y	*n*	Reagents and conditions (first step)	Yield of 3 (%)	α/β ratio
a	Br	H	OBn	OH	2	Et_4NBr, DIPEA, 40 °C, 20 h	76	86:14
b	Br	H	OBn	OH	3	Et_4NBr, DIPEA, 20 °C, 40 h	66	95:05
c	Cl	H	F	F	2	Et_4NBr, DIPEA, 40 °C, 65 h	60	86:14
d	Cl	H	F	F	2	AgTf, Ag_2CO_3, 20 °C, 18 h	50	60:40
e	Cl	H	F	F	3	Et_4NBr, DIPEA, 40 °C, 65 h	86	88:12
f	Cl	F	F	F	3	Et_4NBr, DIPEA, 40 °C, 360 h	33	75:25

Scheme 1.



Scheme 2.

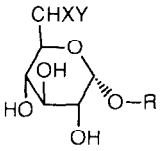
oxalyl chloride/DMSO (Swern oxidation) and then reacted with DAST [26] to give intermediate **5** in 60% yield after flash chromatography purification. In that particular case, boron trichloride could not be used to transform directly intermediate **5** into the expected product **2f**; the methyl glucoside **5** had first to be hydrolyzed and purified before being reacted with thionyl chloride and DMF to make product **2f** suitable for glycosidic bond formation. The final products **3** were then obtained in almost quantitative yield by removal of the benzyl protecting groups by hydrogenation using palladium on charcoal.

The α/β ratios (Scheme 1) have been determined by ^{1}H NMR spectroscopic analysis of the final products **3** in order to avoid any interference with benzylic hydrogen signals. In all cases, the anomeric hydrogen of the α-isomer appears as a doublet between 4.8 and 4.9 ppm with a coupling constant (J_{H1-H2}) of 3.7 Hz, while the anomeric hydrogen of the β-isomer appears between 4.3 and 4.4 ppm with a coupling constant (J_{H1-H2}) of 7.8–8 Hz.

Compounds **1** and **3a–f** were found to be competitive inhibitors of yeast α-glucosidase. Inhibition constants, calculated from a Dixon plot, are given in Table 1. Interestingly, while **3f** (the 6-deoxy-6-difluorinated analogue of **3b**) has no more affinity for the enzyme than its 6-hydroxy counterpart (**3b**), the three 6-deoxy-6-monofluorinated compounds have an affinity for yeast α-glucosidase which is 7- to 25-fold higher than that of the 6-hydroxy derivatives. This result may suggest that the 6-hydroxy group of the glucosides acts as a hydrogen bond acceptor in the active site of yeast α-glucosidase, assuming that both glucosides and 6-deoxy-6-fluorinated glucosides bind identically to the active site. The greater affinity of the 6-deoxy-6-fluorinated glucosides may be further explained by hydrogen-bond formation with an N—H donor group of the protein since an N—H···F bond is stronger than an N—H···O bond, both being exactly the same length [27, 28]. Such

TABLE 1

Effects of fluorine atom(s) in position 6 of α-D-glucopyranosides on yeast α-glucosidase inhibition



X	Y	R	Product	α/β ratio	K_i (μM)	K_{ic}[b] (μM)
H	OH	C_6H_5	**1'**[a]	⩾99:1	850±100	850
H	F	C_6H_5	**1**	⩾99:1	34±3	34
H	OH	$(CH_2)_2SiMe_3$	**3a**	86:14	200±10	170
H	F	$(CH_2)_2SiMe_3$	**3c**	60:40	38±2	23
H	OH	$(CH_2)_3SiMe_3$	**3b**	95:5	42±4	41
H	F	$(CH_2)_3SiMe_3$	**3e**	88:12	6±1	5
F	F	$(CH_2)_3SiMe_3$	**3f**	75:25	95±2	71

[a]Commercially available compound.
[b]K_i values corrected for pure α-anomer of the inhibitor, assuming the β-anomer has no affinity for the enzyme.

N–H···O bonds have been shown previously by crystallography in interactions of glucose-1-phosphate hydroxy groups with the enzyme glycogen phosphorylase-b [14]. Finally, it is worth noting that compound **3e** is one of the most potent inhibitors of yeast α-glucosidase reported so far.

Experimental

General

All reactions were carried out at room temperature. Solvents were dried or distilled before use. Unless otherwise indicated, all reactions were carried out with stirring under argon, and solvents were removed with a rotary evaporator. Proton and fluorine NMR spectra were recorded with either a Varian EM 390 NMR spectrometer (90 MHz) or a Brucker instrument (360 MHz). Chemical shifts are reported downfield from TMS (^{1}H NMR) and hexafluorobenzene (^{19}F).

Materials

Thin layer chromatography (TLC) was undertaken using Kieselgel 60 F_{254} plates (Merck, 0.2-mm layer). Column chromatography was carried out using Kieselgel 60 (230–240 mesh) as the solid phase (E. Merck Laboratories). Methyl-α-D-glucopyranoside, boron trichloride, 2-trimethylsilylethanol, 3-trimethylsilylpropan-1-ol, tetraethylammonium bromide, dimethylaminopyridine, trityl chloride, diisopropylethylamine and silver trifluoromethanesulfonate were purchased from Janssen Chemica. Diethylaminosulfur trifluoride was purchased from Aldrich. Phenyl-α-D-glucopyranoside, *p*-nitrophenyl-α-D-gluco-

pyranoside and α-glucosidase, type III, from yeast were purchased from Sigma Chemical Co., St. Louis, MO, USA.

Assay of α-glucosidase

The enzyme activity was measured at 37 °C in 0.2 M sodium phosphate buffer (pH 6.9) using *p*-nitrophenyl-α-D-glucopyranoside as the substrate, according to Halvorson [18]. Inhibition of α-glucosidase was measured at four concentrations of substrate and five concentrations of inhibitor. K_i values were determined by using a Dixon plot and a computer program developed in-house for linear regression analysis.

*Preparation of 6-deoxy-6-fluoro-2,3,4,-tri-O-benzyl-1-deoxy-1-chloro-α-D-glucopyranose (**2c**)*

Boron trichloride (20 ml of 1 M) in dichloromethane were added dropwise to a stirred solution of 6-deoxy-6-fluoro-2,3,4-tri-*O*-benzyl-methyl-α-D-glucopyranoside (30 mmol, 13.98 g) in 50 ml anhydrous dichloromethane containing 4 Å molecular sieves at 0 °C under argon. The reaction mixture was stirred overnight at 20 °C, quenched with aqueous sodium bicarbonate and extracted with dichloromethane. The organic layers were combined, dried over sodium sulfate, filtered and evaporated to give 14 g of crude product which was used for glucosidic bond formation without further purification. Analysis: ^{1}H NMR $CDCl_3$ δ: 7.3 (m, 15H arom.); 6.0 (d, H_1, $J_{H1-H2}=4$ Hz); 4.5–5.05 (m, 6H benz.); 3.45–4.40 (m, 6H) ppm. ^{19}F NMR $CDCl_3$ δ: 73 (dt, $J_{H5-F}=27$ Hz, $J_{H6-F}=48$ Hz) ppm.

*Preparation of 6-deoxy-6,6-difluoro-2,3,4-tri-O-benzyl-1-O-methyl-α-D-glucopyranoside (**5**)*

DMSO (1.68 ml, 24 mmol) was added slowly to a stirred solution of oxalyl chloride (1.05 ml, 12 mmol) in 10 ml dichloromethane at −78 °C. The reaction mixture was stirred for 10 min at −50 °C, cooled again to −78 °C and a solution of 2,3,4,6-tetra-*O*-benzyl-1-*O*-methyl-α-D-glucopyranoside (4.64 g, 10 mmol) in 15 ml anhydrous dichloromethane was added dropwise. The reaction mixture was stirred at −40 °C for 1 h whereupon 5 ml triethylamine was added dropwise. After 15 min at −40 °C and 1 h at 20 °C, a saturated aqueous ammonium chloride solution was added. The organic phase was separated, the aqueous phase extracted twice with dichloromethane, the organic phases combined, washed with brine, dried over sodium sulfate, filtered and evaporated to give 4.34 g of a brown oil. DAST (3 ml, 24 mmol) was then added dropwise to a stirred solution of this oil dissolved in 40 ml dichloromethane under argon at 20 °C. After stirring at 20 °C for 18 h, the reaction mixture was cooled to 0 °C and quenched with 20 ml methanol. The crude mixture was evaporated to dryness and directly purified by flash chromatography on silica gel giving 2.9 g product (6 mmol, 60%) Analysis: ^{1}H NMR $CDCl_3$ δ: 7.3 (m, 15H arom.); 5.9 (t, CHF_2, $J_{H-F}=54$ Hz); 4.55–5.1 (m, 6H, CH_2 benz.); 4.65 (d, H_1, $J_{H1-H2}=3.5$ Hz); 4.05 (t, H_3); 3.85 (m, H_5); 3.5–3.65 (m, H_2+H_4); 3.35 (s, CH_3) ppm. ^{19}F NMR $CDCl_3$

δ: 29.5 (A part of an ABX system, $J_{FA-H6} = 54$ Hz, $J_{FA-H5} = 8$ Hz, $F_{FA-FB} = 283$ Hz); 27.2 (B part of an ABX system, $J_{FB-H6} = 54$ Hz, $J_{FB-H5} = 19$ Hz) ppm.

*Preparation of 6-deoxy-6,6-difluoro-2,3,4-tri-*O*-benzyl-α-*D*-glucopyranose*

Compound **5** (3.4 mmol, 1.65 g) was dissolved in 35 ml acetic acid and 10 ml 3 N HCl and heated at 90–100 °C for 4.5 h. The crude mixture was diluted by the addition of 100 ml water and extracted four times with ethyl acetate. The combined organic phases were washed with saturated aqueous bicarbonate (4×) and brine, dried over sodium sulfate, filtered and evaporated to give 1.6 g of a dark brown residue which was purified by flash chromatography on silica gel giving 770 mg product (48% yield). Analysis: ^{1}H NMR $CDCl_3$ δ: 7.3 (m, 15H arom.); 5.9 (t, H_6, $J_{H6-F} = 54$ Hz); 5.15 (d, H_1, $J_{H1-H2} = 3.5$ Hz); 4.4–5 (m 6H benz.); 3.3–4.35 (m, 5H); 3.2 (m, OH) ppm.

*Preparation of 6-deoxy-6,6-difluoro-2,3,4-tri-*O*-benzyl-1-deoxy-1-chloro-α-*D*-glucopyranose (**2f**)*

Thionyl chloride (10 mmol, 1.35 g) was added to a stirred solution of 3 ml DMF in 30 ml anhydrous dichloromethane under argon at 0 °C. After 20 min, 6-deoxy-6-6-difluoro-2,3,4-tri-*O*-benzyl-α-D-glucopyranose (4.7 g, 10 mmol) dissolved in 20 ml dichloromethane was added to the reaction mixture which was stirred for 45 min at 20 °C, quenched with saturated aqueous bicarbonate and extracted with dichloromethane. The organic phases were combined, washed with water and brine, dried over sodium sulfate, filtered and evaporated to give 4.50 g crude product which was used for glucosidic bond formation without further purification. Analysis: ^{1}H NMR $CDCl_3$ δ: 7.3 (m, 15H arom.); 6.05 (d, H_1, $J_{H1-H2} = 4$ Hz); 5.9 (t, H_6, $J_{H6-F} = 54$ Hz); 4.6–5.1 (6H benz.); 3.25–4.3 (m, 4H) ppm.

*Glucosidic bond formation (typical example): preparation of 6-deoxy-6-fluoro-2,3,4-tri-*O*-benzyl-(3-trimethylsilyl)propyl-α-*D*-glucopyranoside*

A mixture consisting of 0.73 mmol (346 mg) **2c**, 2 mmol (420 mg) tetraethylammonium bromide, 1.5 mmol (193 mg) diisopropyl ethylamine and 1.5 mmol (198 mg) 3-trimethylsilylpropan-6-ol in 8 ml anhydrous dichloromethane, containing 4 Å molecular sieves, was stirred for 65 h at 40 °C. The reaction mixture was evaporated under reduced pressure and the residue extracted with ethyl acetate, washed with water and brine, dried over sodium sulfate, filtered, evaporated and purified by flash chromatography on silica gel using petroleum ether and increasing amounts of ethyl acetate as solvents to give 340 mg of expected product (86%). Analysis: ^{1}H NMR $CDCl_3$ δ: 7.3 (m, 15H arom.); 4.5 (m, 6H benz. + H_1); 3.4–4.5 (m, 8H); 1.7 (m, $2H_2$); 0.5 (m, CH_2SiMe_3); 0.0 (s, 9H, $SiMe_3$) ppm. ^{19}F NMR $CDCl_3$ δ: −70.5 (dt, $J_{F-H6} = 48$ Hz, $J_{F-H5} = 27$ Hz) ppm.

Removal of the O-*benzyl protecting groups (typical procedure: synthesis of 6-deoxy-6-fluoro-1-*O-*(3-trimethylsilyl)propyl-α-*D-*glucopyranose*

6-Deoxy-6-fluoro-2,3,4-tri-*O*-benzyl-1-*O*-(3-trimethylsilyl)propyl-α-D-glucopyranose (0.6 mmol, 340 mg) was dissolved in 9 ml anhydrous THF and stirred overnight under hydrogen at atmospheric pressure in the presence of 40 mg Pd/C. The reaction mixture was filtered over Celite, evaporated and recrystallized from ether/cyclohexane to give 127 mg product (71%).

Analyses of the various products obtained were as follows:

(2-Trimethylsilyl)ethyl-α-D-glucopyranoside (**3a**): ^{1}H NMR CD_3CN δ: 4.8 (d, $H_{1\alpha}$, $J_{H1-H2}=3.8$ Hz); 4.3 (d, $H_{1\beta}$, $J_{H1-H2}=7.7$ Hz, 14% of $H_{1\alpha}$); 3.8–3.9 (m, H_5); 3.6–3.8 (m, $2H_6$); 3.5–3.6 (m, H_2, H_3 and H_4); 3.2–3.4 (m, $2H_{1'}$); 1.0 (m, $2H_{2'}$); 0.1 (s, 9H, $SiMe_3$) ppm. Melting point, 106 °C. Anal.: Calcd.: C, 47.12; H, 8.63%. Found: C, 46.95; H, 8.25%.

(3-Trimethylsilyl)propyl-α-D-glucopyranoside (**3b**): ^{1}H NMR (CD_3CN δ: 4.82 (d, H_1 (α), $J_{H1-H2}=3.8$ Hz); 4.3 (d, H_1 (β), 5%, $J_{H1-H2}=7.8$-Hz); 2.8–3.8 (m, 12H); 1.7 (m, $2H_{2'}$); 0.6 (m $2H_{3'}$); 0.1 (s, 9H, $SiMe_3$) ppm. Melting point, 111 °C. Anal.: Calcd. C, 48.96; H, 8.90%. Found, C, 48.96: H, 8.96%.

6-Deoxy-6-fluoro(2-trimethylsilyl)ethyl-α-D-glucopyranoside (**3c**): ^{1}H NMR CD_3CN δ: 4.85 (d, $H_{1\alpha}$ (60%), $J_{H1-H2}=3.8$ Hz); 4.3 (d, $H_{1\beta}$ (40%), $J_{H1-H2}=7.7$ Hz); 4.5–4.7 (m, $2H_6$); 3.3–4 (m, 9H); 1 (m, $2H_{2'}$); 0.05 (s, 9H, $SiMe_3$) ppm. ^{19}F NMR CD_3CN δ: −69.9 (dt, F_b (40%), $J_{Fb-H6}=48$ Hz, $J_{F-H5}=25$ Hz); −70.2 (dt, F_a (60%), $J_{Fa-H6}=48$ Hz, $J_{Fa-H5}=27$ Hz) ppm. Melting point, 101 °C. Anal.: Calcd.: C, 46.79; H, 8.21%. Found: C, 47.03; H, 8.63%.

6-Deoxy-6-fluoro-(3-trimethylsilyl)propyl-α-D-glucopyranoside (**3e**): ^{1}H NMR CD_3CN δ: 4.85 (d, H_1 (α), $J_{H1-H2}=3.7$ Hz), 4.62 (m, $2H_6$, $J_{H6A-H6B}=10.5$ Hz, $J_{H6A-H5}=2.1$ Hz, $J_{H6B-H5}=3.8$ Hz, $J_{H6-F}=48$ Hz); 4.31 (d, H_1 (β) (13%), $J_{H1-H2}=7.8$ Hz); 3.3–3.9 (m, 9H); 1.65 (m, $2H_{2'}$); 0.6 (m, $2H_{3'}$), 0.1 (s, 9H, $SiMe_3$) ppm. ^{19}F NMR CD_3CN α-isomer (87%) δ: −70.2 (dt, $J_{F-H6}=98$ Hz, $J_{F-H5}=27$ Hz). β-isomer (13%): −69.9 (dt, $J_{F-H6}=48$ Hz, $J_{F-H5}=26$ Hz) ppm. Melting point, 91 °C. Anal.: Calcd.: C, 48.62; H, 8.50%. Found: C, 49.17; H, 8.56%.

6-Deoxy-6,6-difluoro(3-trimethylsilyl)propyl-α-D-glucopyranoside (**3f**): ^{1}H NMR CD_3CN δ: 6.1 (t, H_6, $J_{H-F}=46$ Hz); 4.9 (d, H_1 (α), $J_{H1-H2}=3.7$ Hz); 4.35 (d, H_1 (β), 25% of H_1 (α) signal, $J_{H1-H2}=7.8$ Hz); 3.1–3.9 (m, 9H); 1.65 (m, $2H_{2'}$); 0.6 (m, $2H_{3'}$); 0.1 (s, 9H, $SiMe_3$) ppm. ^{19}F NMR CD_3CN α-isomer (75%) δ: 31.4 (A part of an ABMX system, $J_{F-F}=283$ Hz, $J_{H6-HA}=54$ Hz, $J_{H5-FA}=8$ Hz); 28.1 (B part of an ABX system, $J_{H6-FB}=54$ Hz, $J_{H5-FB}=19$ Hz) ppm. Melting point, 103–104 °C. Anal.: Calcd.: C, 45.84; H, 7.69%. Found: C, 45.82; H, 7.62%.

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