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Note

Synthesis of 2-deoxy-2-fluoro-glucotropaeolin, a thioglucosidase inhibitor

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Abstract

S_N2-Displacement of the anomeric bromine atom in 3,4,6-tri-*O*-acetyl-2-deoxy-2-fluoro-α-D-glucopyranosyl bromide with the tetrabutylammonium salt of triphenylmethanethiol afforded the corresponding trityl 1-thio-β-D-glucoside which led to the fully protected *S*-acetyl-2-fluoro-1-thio-β-D-glucose derivative, and then to the free thiol **5** by selective *S*-deacetylation at low temperature. 2-Fluoro-glucotropaeolin (**1**) was obtained by a conventional procedure from **5**.

Keywords: Glucosinolates; Glucotropaeolin; Thioglycosides; 2-Deoxy-2-fluoro-glycosides; Myrosinase; Thioglucosidase inhibitor

2-Deoxy-2-fluoro-glycosides have recently been found particularly useful in the determination of the molecular mechanism of *O*-glycosyl hydrolases by trapping the glycosyl-enzyme intermediates [1]. Thioglucosidase (thioglycoside glucohydrolase, EC 3.2.3.1 myrosinase) is the only known glycosidase able to degrade glucosinolates, which constitute a group of anomeric thiohydroxymoyl derivatives of 1-thio-β-D-glucopyranose [2]. The amino acid sequence of the protein has been shown to display strong similarities with the glycosyl hydrolases (EC 3.2.1–3.2.3) family [3], suggesting that the enzyme probably shares the same stereochemical course and mechanism of hydrolysis. In order to elucidate the mechanism of action of thioglucosidase, which is still a matter of debate [4,5], the synthesis of 2-deoxy-2-fluoro-glucotropaeolin **1** (Scheme 1), mechanism-based inhibitor of the enzyme has been investigated.

The strategy used for the synthesis of **1** involved the preparation of the 2-deoxy-2-fluoro-1-thio-β-D-glucopyranose **5** and its coupling to phenylacethydroximoyl chloride **6**, followed by *O*-sulfation, according to the general procedure for the preparation of synthetic glucosinolates [6]. A first attempt to synthesize **5** starting from 3,4,6-tri-*O*-acetyl-2-deoxy-2-fluoro-α-D-glucopyranosyl bromide **2** [7] and thiourea failed [8,9]. This prompted us to use an alternative procedure, developed in our laboratory [10] for introducing a thiol group at the anomeric position of the sugar residue. Displacement of the

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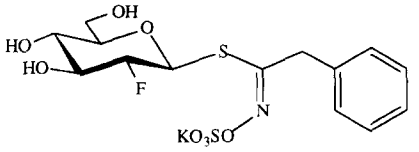

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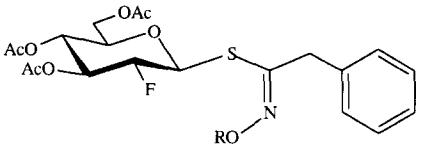

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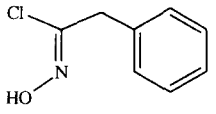
8 R = OSO_3K



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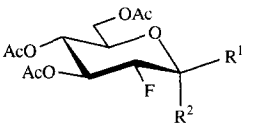

	R^1	R^2
2	H	Br
3	$SCPh_3$	H
4	SAc	H
5	SH	H

Scheme 1.

anomeric bromine atom in **2** with the tetrabutylammonium salt of triphenylmethanethiol in toluene then furnished **3**, albeit in 21% yield. Reaction of thioglycoside **3** with phenylmercury(II) acetate, followed by treatment with hydrogen sulfide in the presence of pyridine and acetic anhydride gave **4** in high yield. The structure of **4** was confirmed by its ^1H NMR spectrum.

The selective *S*-deacylation of **4** was performed with an equivalent amount of sodium methoxide in methanol at −50 °C to give **5**, and we noticed that the conditions of neutralization with the acidic resin appeared to be crucial in order to avoid the formation of the symmetrical disulfide. Coupling of thiol **5** with the hydroximoyl chloride **6** [11], then *O*-sulfation of the resulting thiohydroximate **7** using the pyridine–sulfur trioxide complex followed by formation of the potassium salt with potassium hydrogencarbonate, gave the protected glucosinolate **8**. *O*-Deacylation with potassium methoxide in methanol and LC purification on a C-18 cartridge yielded the 2-fluoro derivative of glucotropaeolin **1**, which satisfied analysis by ^1H, ^{13}C NMR and mass spectrometry.

Glucosinolate analog **1** has been successfully tested as an enzyme-activated inhibitor of *Sinapis alba* thioglucosidase [12]. As could be expected from the results obtained for several β-glycosidases by Withers et al. [1], incubation of the enzyme with **1** resulted in time-dependent inactivation through stabilization and trapping of the glycosyl-enzyme intermediate. The full results of the biochemical experiments have been published elsewhere [13].

1. Experimental

General methods.—Melting points were measured on a Büchi 535 apparatus and are uncorrected. Optical rotations were obtained using a Perkin Elmer 261 polarimeter at ambient temperature. ^1H (300.133 MHz) and ^{13}C (75.468 MHz) NMR spectra were recorded with a Bruker AC 300 spectrometer. Low resolution mass spectra were recorded on a Nermag R 10 10 C spectrometer using chemical ionization (CIMS) in positive mode or fast atom bombardment (FABMS) in negative mode. Flash-column chromatography was performed on Silica Gel 60 (230–400 mesh, E. Merck). TLC was performed on Silica Gel $60F_{254}$ (E. Merck) with detection by UV light or by charring with diluted H_2SO_4.

3,4,6-Tri-O-acetyl-1-S-acetyl-2-deoxy-2-fluoro-1-thio-β-D-glucopyranose (**4**).—A soln of tetrabutylammonium hydroxide (2.8 mL, 25% in MeOH) and triphenylmethanethiol (0.61 g, 2.21 mmol) in MeOH (3 mL) was coevaporated with dry toluene (4 × 3 mL). To the resulting thiolate was added a soln of bromide **2** [7] (0.42 g, 1.13 mmol) in dry toluene (6 mL). The reaction mixture was stirred at room temperature overnight, then concentrated under reduced pressure. Flash-column chromatography (1:20 EtOAc–toluene) of the residue gave triphenylmethyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-fluoro-1-thio-β-D-glucopyranoside (**3**, 134 mg, 21%). No satisfactory analysis could be obtained for this compound, which was used in the next step without characterization. Phenylmercury(II) acetate (63 mg, 0.187 mmol) was added to a soln of the *S*-trityl glycoside **3** (100 mg, 0.177 mmol) in CH_2Cl_2 (0.6 mL) and MeOH (2.2 mL). The reaction mixture was stirred for 4 h at room

temperature then concentrated under reduced pressure. The residue, dried through coevaporation with toluene, was dissolved in CH_2Cl_2 (2.6 mL), then pyridine (1 mL) and Ac_2O (1 mL) were added. Hydrogen sulfide was bubbled into the soln for 25 min, and the resulting cloudy mixture was stirred at room temperature for 1 h, and concentrated under reduced pressure. Flash-column chromatography (1:20 EtOAc–toluene) of the residue gave **4** (62 mg, 96%); mp 118–120 °C (from Et_2O); $[\alpha]_D^{20}$ +11.5° (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 5.33 (dt, 1 H, H-3), 5.27 (dd, 1 H, H-1), 4.99 (t, 1 H, H-4), 4.37 (ddd, 1 H, H-2), 4.21 (dd, 1 H, H-6a), 4.04 (dd, 1 H, H-6b), 3.81 (ddd, 1 H, H-5), 2.37 (s, 3 H, SAc), 2.02–1.99 (3 s, each 3 H, 3 × OAc); $J_{1,2}$ 10.2, $J_{2,3}$ 8.8, $J_{3,4}$ 9.8, $J_{4,5}$ 9.8, $J_{5,6a}$ 4.6, $J_{5,6b}$ 2.2, $J_{6a,6b}$ 12.5, $J_{1,F}$ 2.0, $J_{2,F}$ 49.6, $J_{3,F}$ 14.1 Hz; ^{13}C NMR ($CDCl_3$): δ 191.2 ($SCOCH_3$), 170.2, 169.5, 169.2 (3 × $OCOCH_3$), 87.0 (d, C-2), 79.3 (d, C-1), 76.1 (C-5), 73.7 (d, C-3), 67.5 (d, C-4), 61.4 (C-6), 30.5 ($SCOCH_3$), 20.4, 20.3, 20.2 (3 × $OCOCH_3$), $J_{1,F}$ 24, $J_{2,F}$ 191, $J_{3,F}$ 20, $J_{4,F}$ 7 Hz. Anal. Calcd for $C_{14}H_{19}FO_8S$: C, 45.89; H, 5.22; F, 5.18; S, 8.75. Found: C, 46.14; H, 5.38; F, 5.79; S, 8.80.

3,4,6-Tri-O-acetyl-2-deoxy-2-fluoro-1-thio-β-D-glucopyranose (**5**).—To a cooled (−50 °C) soln of **4** (150 mg, 0.41 mmol) in MeOH (20 mL) and CH_2Cl_2 (5 mL) was added an equivalent amount of NaOMe (0.41 mL, 1 M in MeOH). After 2 h, Amberlite® IRN 77 (H^+ form) was added to neutralize the soln, and stirring was continued for 1 h at −50 °C. The resin was quickly filtered off, and the filtrate concentrated under reduced pressure. Recrystallization from MeOH gave **5** (120 mg, 90%); mp 120–122 °C; $[\alpha]_D^{20}$ +30° (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 5.22 (dt, 1 H, H-3), 4.99 (t, 1 H, H-4), 4.59 (m, 1 H, H-1), 4.18 (dd, 1 H, H-6a), 4.16 (m, 1 H, H-2), 4.05 (dd, 1 H, H-6b), 3.67 (ddd, 1 H, H-5), 2.32 (d, 1 H, SH), 2.02–1.99 (3s, each 3 H, 3 × OAc); $J_{1,2}$ 8.9, $J_{2,3}$ 9.2, $J_{3,4}$ 9.8, $J_{4,5}$ 9.8, $J_{5,6a}$ 4.9, $J_{5,6b}$ 2.2, $J_{6a,6b}$ 12.5, $J_{1,SH}$ 8.1, $J_{2,F}$ 49.1, $J_{3,F}$ 13.8 Hz; ^{13}C NMR ($CDCl_3$): δ 170.5, 169.9, 169.4 (3 × $OCOCH_3$), 91.6 (d, C-2), 79.1 (d, C-1), 76.4 (C-5), 73.6 (d, C-3), 67.9 (d, C-4), 61.9 (C-6), 20.7, 20.6, 20.5 (3 × $OCOCH_3$), $J_{1,F}$ 25, $J_{2,F}$ 192, $J_{3,F}$ 20, $J_{4,F}$ 7 Hz. Anal. Calcd for $C_{12}H_{17}FO_7S$: C, 44.44; H, 5.28; F, 5.85; S, 9.88. Found: C, 44.31; H, 5.22; F, 5.89; S, 9.38.

S-(3,4,6-Tri-O-acetyl-2-deoxy-2-fluoro-β-D-glucopyranosyl) phenylacetothiohydroximate (**7**).—To a suspension of *N*-chlorosuccinimide (38 mg, 0.3 mmol) in CH_2Cl_2 (2 mL) was added pyridine (0.1 mL), then phenylacetaldoxime [11] (40 mg, 0.3 mmol). The mixture was stirred for 2.5 h at room temperature, then thiol **5** (50 mg, 0.154 mmol) was added, and stirring was continued for 1.5 h. After concentration under reduced pressure, flash-column chromatography (1:3 EtOAc–cyclohexane) of the residue gave **7** (50 mg, 71%); $[\alpha]_D^{20}$ −7° (*c* 1.0, MeOH); ^{1}H NMR ($CDCl_3$): δ 8.30 (bs, 1 H, NOH), 7.40–7.25 (m, 5 H, aromatic Hs), 5.11 (dt, 1 H, H-3), 4.94 (t, 1 H, H-4), 4.76 (dd, 1 H, H-1), 4.27 (ddd, 1 H, H-2), 4.12 (dd, 1 H, H-6a), 3.98 (dd, 1 H, H-6b), 3.96 (s, 2 H, CH_2Ph) 3.47 (ddd, 1 H, H-5), 2.02–1.99 (3s, each 3 H, 3 × OAc); $J_{1,2}$ 9.9, $J_{2,3}$ 9.1, $J_{3,4}$ 9.1, $J_{4,5}$ 9.8, $J_{5,6a}$ 5.5, $J_{5,6b}$ 2.3, $J_{6a,6b}$ 12.4, $J_{1,F}$ 1.6, $J_{2,F}$ 49.7, $J_{3,F}$ 14.8 Hz; ^{13}C NMR ($CDCl_3$): δ 170.2, 169.7, 169.1 (3 × $OCOCH_3$), 150.7 (C=N), 135.3, 128.8, 127.8, 127.3 (aromatic Cs), 88.0 (d, $J_{2,F}$ 194 Hz, C-2), 78.3 (d, $J_{1,F}$ 23 Hz, C-1), 75.4 (C-5), 73.4 (d, $J_{3,F}$ 20 Hz, C-3), 67.6 (d, $J_{4,F}$ 7 Hz, C-4), 61.8 (C-6), 38.9 (CH_2Ph), 20.4, 20.3, 20.2 (3 × $OCOCH_3$). CIMS: *m/z* 458 $(M + H)^+$.

S-(2-Deoxy-2-fluoro-β-D-glucopyranosyl) O-(potassium sulfonato) phenylacetothiohydroximate (2-deoxy-2-fluoro-glucotropaeolin, **1**).—The pyridine–sulfur trioxide complex was prepared immediately before use by carefully adding dry pyridine (385 μL) to a soln of chlorosulfonic acid (83 μL, 1.22 mmol) in dry CH_2Cl_2 (1.65 mL). The resulting suspension was stirred for 25 min, then a soln of **7** (55 mg, 0.12 mmol) in CH_2Cl_2 (1 mL) was added, and stirring was continued for 2 d under Ar. The potassium salt was obtained by adding a soln of $KHCO_3$ (220 mg, 2.2 mmol) in water (2.75 mL). The mixture was stirred for 30 min and then diluted with $CHCl_3$, the organic extracts being washed two times with H_2O, dried ($MgSO_4$), and concentrated under reduced pressure. Flash-column chromatography (CH_2Cl_2, then 4:1 CH_2Cl_2–MeOH) of the residue gave *S*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-fluoro-β-D-glucopyranosyl) *O*-(potassium sulfonato) phenylacetothiohydroximate **8** (2-deoxy-2-fluoro-glucotropaeolin triacetate, 61 mg, 88%); $[\alpha]_D^{20}$ −4° (*c* 1.2, MeOH); ^{1}H NMR (9:1 CD_3COCD_3–CD_3OD): δ 7.45–7.25 (m, 5 H, aromatic Hs), 5.25 (dt, 1 H, H-3), 5.14 (dd, 1 H, H-1), 4.97 (t, 1 H, H-4), 4.40 (ddd, 1 H, H-2), 4.13 (m, 3 H, H-6a, CH_2Ph), 3.94 (dd, 1 H, H-6b), 3.88 (ddd, 1 H, H-5), 2.01–1.99 (3s, each 3 H, 3 × OAc); $J_{1,2}$ 9.8, $J_{2,3}$ 8.7, $J_{3,4}$ 9.8, $J_{4,5}$ 9.8, $J_{5,6b}$ 2.3, $J_{6a,6b}$ 12.3, $J_{1,F}$ 1.7, $J_{2,F}$ 50.0, $J_{3,F}$ 14.7 Hz; ^{13}C NMR (9:1 CD_3COCD_3–CD_3OD): δ 171.4, 170.8, 170.6 (3 × $OCOCH_3$), 157.6 (C=N), 137.1, 130.3, 129.6, 128.6 (aromatic Cs), 89.8 (d, C-2), 79.7 (d, C-1), 76.8 (C-5), 74.7 (d, C-3), 69.1 (d, C-4), 63.2 (C-6), 39.5

(CH_2Ph), 21.1, 20.9 ($3 \times OCOCH_3$), $J_{1,F}$ 24, $J_{2,F}$ 191, $J_{3,F}$ 19, $J_{4,F}$ 7 Hz.

To a soln of **8** (25 mg, 43 μmol) in MeOH (5 mL) was added potassium methoxide (20 μL, 0.8 M in MeOH). The soln was stirred overnight, filtrated through cotton wool, and the filtrate concentrated under reduced pressure. The residue was dissolved in H_2O, then purified by LC using a Sep-Pak Plus® C-18 cartridge and elution with water. The appropriate fractions were pooled, then freeze-dried to give **1** (11 mg, 56%); $[\alpha]_D^{20}$ −10.5° (*c* 0.9, H_2O); ^1H NMR (D_2O): δ 7.35–7.20 (m, 5 H, aromatic Hs), 4.86 (dd, 1 H, H-1), 4.09 (ddd, 1 H, H-2), 4.05 (bs, 2 H, CH_2Ph), 3.53 (m, 3 H, H-3, H-6a, H-6b), 3.37 (t, 1 H, H-4), 3.18 (ddd, 1 H, H-5); $J_{1,2}$ 9.7, $J_{2,3}$ 8.5, $J_{3,4}$ 9.5, $J_{4,5}$ 9.5, $J_{1,F}$ 1.5, $J_{2,F}$ 50 Hz; ^{13}C NMR (D_2O): δ 163.2 (C=N), 136.4, 130.7, 139.6, 129.1 (aromatic Cs), 91.6 (d, C-2), 81.3 (C-5), 79.7 (d, C-1), 76.6 (d, C-3), 69.8 (d, C-4), 61.5 (C-6), 39.6 (CH_2Ph), $J_{1,F}$ 23, $J_{2,F}$ 189, $J_{3,F}$ 17, $J_{4,F}$ 9 Hz. FABMS: *m/z* 409 $(M-H)^-$.

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