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The synthesis of diosgenyl 2-amino-2-deoxy-β-D-glucopyranoside hydrochloride

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Abstract

The *N*-trifluoroacetyl- and *N*-tetrachlorophthaloyl-protected bromide of D-glucosamine has been used for the first time as a glycosyl donor for the glycosylation of diosgenin [(25*R*)-spirost-5-en-3β-ol]. Both 1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-trifluoroacetamido-β-D-glucopyranoside and 1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-tetrachlorophthalimido-α,β-D-glucopyranoside were transformed into the appropriate glycosyl bromides. These reacted with diosgenin under mild conditions, using silver triflate as a promoter, and gave the corresponding protected diosgenyl glycosides. Each was deprotected to give diosgenyl 2-amino-2-deoxy-β-D-glucopyranoside hydrochloride. The structures of the new glycosides were established by ^{1}H NMR spectroscopy.

Keywords: 2-Amino-2-deoxy-D-glucose derivatives; D-Glucosamine derivatives; Diosgenin; (25*R*)-Spirost-5-en-3β-ol; Glycosylation; Deprotection

Saponins are glycosides distributed widely in plants and in some marine organisms [1,2]. They are classified as steroid or triterpenoid glycosides depending upon the nature of the aglycone. The carbohydrate residue is attached covalently to the sapogenin backbone, for example to diosgenin. The diosgenyl glycosides may exert a large variety of biological functions, some of which can be ascribed to the sapogenin moiety and some to the carbohydrate residue [3–6]. Some of the diosgenyl glycosides exhibit a wide spectrum of biological activities and have been used in materia medica to treat malaria, helminthes infections, and snake bites. Also it has been found that the crude extract of some plants containing the diosgenin glycosides, especially in the aerial portion, has antineoplastic properties against several strains of human cancer cells [7–9]. The extreme difficulties associated with the purification of saponins from natural sources force the synthesis of these types of glycosides [10–14].

We now report the synthesis of some diosgenyl glycosides with D-glucosamine derivatives as the carbohydrate residue. The synthetic strategy is based on the coupling of

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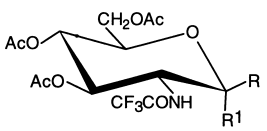
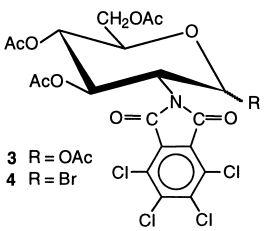
the protected D-glucosaminyl halide with diosgenin, separation of these products, and removal of the protecting groups [15].

Compound **1** was obtained from commercially available D-glucosamine hydrochloride in four steps: (i) the amino group was first *N*-protected with *p*-anisaldehyde in aqueous 1 M sodium hydroxide solution; (ii) this product was acetylated with acetic anhydride in pyridine; (iii) the *N*-*p*-methoxybenzylidene group was removed using 5 M hydrochloric acid in warm acetone; (iv) finally, *N*-acetylation of the 2-amino sugar derivative with trifluoroacetic anhydride in a dichloromethane–pyridine mixture gave **1** [16,17].



1 R = OAc, R1 = H
2 R = H, R1 = Br



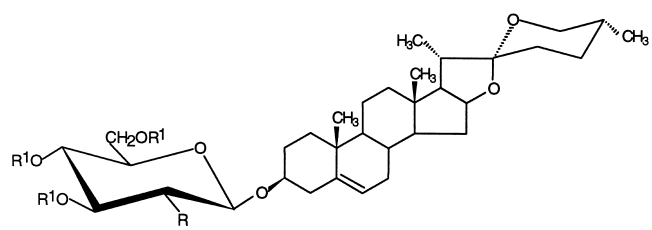
3 R = OAc
4 R = Br

D-Glucosamine hydrochloride was also converted into **3** as follows: (i) neutralization of the hydrochloride by a stoichiometric amount of 1 M methanolic sodium methoxide solution, with immediate acylation of the 2-amino group with tetrachlorophthalic anhydride; (ii) O-acetylation, after evaporation of methanol, of the crude product with acetic anhydride in pyridine furnished the NTCP-protected per-*O*-acetyl derivative **3**, as an anomeric mixture, in good yield [18–20].

Treatment of the *N*-protected per-*O*-acetylated derivative **1** and **3** with excess of titanium tetrabromide [21,22] in 10:1 dichloromethane–ethyl acetate resulted in the glucosyl bromides **2** and **4**, respectively. All new sugar derivatives were characterized by ^{1}H NMR (400 MHz) spectroscopy, and known compounds were identified by comparing their melting points and $[\alpha]_D$ values with those in the literature.

Both glycosylation of 3,4,6-tri-*O*-acetyl-2-deoxy-2-trifluoroacetamido-α-D-glucopyranosyl bromide (**2**) and 3,4,6-tri-*O*-acetyl-2-deoxy-2-tetrachlorophthalimido-α,β-D-glucopyranosyl bromide (**4**) with diosgenin in dichloromethane under nitrogen at room temperature in the presence of silver triflate [23,24] gave the glycosides **5** and **6**, respectively, in ~65% yield. After completion of the reaction, as determined by thin-layer chromatography, the mixture was diluted with chloroform, filtered, and worked up in the usual manner. The reaction is stereospecific, and the products were shown by ^{1}H NMR spectroscopy to have the β configuration.

The hydrolysis of **5** with 1 M sodium methoxide in methanol gave the partially deprotected glycoside **7**. On the other hand, the treatment of **5** in acetone with 1 M aqueous sodium hydroxide, followed by neutralization with Dowex-50W (H^+) ion-exchange resin, gave the fully deblocked compound **8**, which was isolated as the hydrochloride.



5 R = NHCOCF3, R1 = Ac
6 R = NTCP, R1 = Ac
7 R = NHCOCF3, R1 = H
8 R = NH2·HCl, R1 = H

Mild treatment of the glycoside **6** with 1,2-diaminoethane produced the compound with free amino and hydroxy groups in the sugar moiety, which was finally isolated as hydrochloride **8**. The structures of **5**–**8** were established on the basis of the ^{1}H NMR (400 MHz) spectroscopy (Table 1). Compound **8** Anal. Calcd for $C_{33}H_{53}NO_7 \cdot HCl \cdot 1.5H_2O$: C, 62.00; H, 8.99; N, 2.19. Found C, 61.94; H, 8.67; N, 1.94%.

The influence of diosgenyl 2-amino-2-deoxy-β-D-glucopyranoside hydrochloride (**8**) on lymphocytes, isolated from the blood of both patients and healthy individuals, was evaluated. Patients suffering from cancer (lymphoma) and were treated with 2-chloro-2′-deoxyadenosine (2-CDA). Compound **8**

Table 1
Selected ^{1}H NMR chemical shifts (δ), coupling constants (J) and polarimetric data ($[\alpha]_D^{20}$) [a]

Compound	H-1	H-2	H-3	H-4	H-5	H-6	Other protons	J (Hz)	$[\alpha]_D^{20}$
5 in $CDCl_3$	4.78 (d)	3.88 (dd)	5.32 (dd)	5.10 (dd)	3.72 (m)	4.28 (dd) 4.12 (dd)	6.43 (d), NH 2.10, 6 H, OAc 2.15, 3 H, OAc	$J_{1,2} = J_{2,3} = 10$ $J_{3,4} = J_{4,5} = 10$ $J_{5,6} = 4.9$ Hz; $J_{5,6'} = 3$ $J_{gem} = 12$	$-74°$ (c 0.6; CH_3OH)
	Diosgenyl protons: 5.33 (m), $C_{(6)}$–H; 4.40 (dd), $C_{(3)}$–H; 3.48 (m), $C_{(16)}$–H; 3.46 (m), $C_{(26)}$–$H_{(e)}$; 3.37 (m), $C_{(26)}$–$H_{(a)}$; 2.20 (m), $C_{(15)}$–2H; 1.28 (m), $C_{(2)}$–2H; 0.98 (s), CH_3; 0.96 (d), CH_3; 0.78 (s), CH_3; 0.75 (d) CH_3								
6 in $CDCl_3$	5.47 (d)	4.29 (dd)	5.67 (dd)	5.18 (dd)	3.82 (m)	4.33 (dd) 4.15 (dd)	1.90, 3 H, OAc 2.04, 3 H, OAc 2.10, 3 H, OAc	$J_{1,2} = 8.4$ $J_{2,3} = 10.8$ $J_{3,4} = 10.4$; $J_{4,5} = 8.8$ $J_{5,6} = 4.8$; $J_{5,6'} = 2.4$	12° (c 0.5; CH_3Cl)
	Diosgenyl protons: 5.29 (m), $C_{(6)}$–H; 4.40 (dd), $C_{(3)}$–H; 3.48 (m), $C_{(26)}$–$H_{(e)}$; 3.37 (t), $C_{(26)}$–$H_{(a)}$; 2.20 (m), $C_{(15)}$–2H; 1.27 (m), $C_{(2)}$–2H; 0.97 (d), CH_3; 0.94 (s), CH_3; 0.79 (d), CH_3; 0.77 (s) CH_3								
7 in $(CD_3)_2SO$	4.50 (d)		3.10–3.38 (m)			4.28 (m) 3.48 (m)		$J_{1,2} = 9.2$	$-53°$ (c 0.8; CH_3OH)
	Diosgenyl protons: 5.30 (m), $C_{(6)}$–H; 4.28 (dd), $C_{(3)}$–H; 2.28 (m), $C_{(15)}$–H′; 1.90 (m), $C_{(15)}$–H; 0.92 (s), CH_3; 0.90 (d), CH_3; 0.76 (s), CH_3; 0.72 (d) CH_3								
8 in 1:1 $CDCl_3/CD_3OD$	4.37 (d)	2.62 (dd)	3.30 (m)	3.33 (dd)	3.26 (m)	3.84 (m) 3.71 (m)		$J_{1,2} = 8.1$; $J_{2,3} = 9.3$ $J_{3,4} = J_{4,5} = 8.3$ $J_{5,6} = 5$; $J_{5,6'} = 2.3$ $J_{gem} = 12$	$-56°$ (c 0.4; 1:1 $CHCl_3$–CH_3OH)
	Diosgenyl protons: 5.35 (m), $C_{(6)}$–H; 4.40 (dd), $C_{(3)}$–H; 3.58 (m), $C_{(16)}$–H; 3.45 (m), $C_{(26)}$–$H_{(e)}$; 3.32 (m), $C_{(26)}$–$H_{(a)}$; 2.28 (m), $C_{(15)}$–H′; 1.90 (m), $C_{(15)}$–H; 0.92 (s), CH_3; 0.90 (d), CH_3; 0.76 (s), CH_3; 0.72 (d) CH_3								

[a] Spectra were recorded on a Varian 400 MHz spectrometer with Me_4Si as the internal standard.

showed a cytotoxic effect against lymphocytes isolated from the patients' blood, as tested by Trypane Blue exclusion [25]. In addition, **8** was shown to enhance the cytostatic effect of 2-CDA, and it significantly reduced (from 20 to 30%) the number of lymphatic cancer cells. Compound **8** was shown to be more effective in limiting the number of cancer cells than cells of healthy individuals.

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