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Synthesis and induction of apoptosis in B cell chronic leukemia by diosgenyl 2-amino-2-deoxy-β-D-glucopyranoside hydrochloride and its derivatives

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Abstract

2-Acetamido-2-deoxy-D-glucose hydrochloride (D-glucosamine hydrochloride) has been used for the preparation of 1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-trifluoroacetamido-β- (**4**) and 2-tetrachlorophthalimido-α,β-D-glucopyranose (**6**), which have been transformed into the appropriate bromides and the chloride. Both bromo and chloro sugars were used as a glycosyl donors for the glycosylation of diosgenin [(25*R*)-spirost-5-en-3β-ol]. These condensations were conducted under mild conditions, using silver triflate as a promoter, and gave diosgenyl glycosides **9** and **12**. Each of them was converted into diosgenyl 2-amino-2-deoxy-β-D-glucopyranoside hydrochloride (**11**) and *N*-acylamido derivatives. The structures of all new glycosides were established by ^{1}H and ^{13}C NMR spectroscopy. These diosgenyl glycosides are the first saponins containing the D-glucosamine residue that have been synthesized. These compounds show promising antitumor activities. The synthetic saponins increase the number of apoptotic B cells, in combination with cladribine (2-CdA), that are isolated from chronic lymphotic leukemia (B-CLL) patients. © 2002 Published by Elsevier Science Ltd.

Keywords: 2-Acetamido-2-deoxy-D-glucose; D-Glucosamine; Diosgenin; (25*R*)-Spirost-5-en-3β-ol; Diosgenyl glycosides; Trifluoroacetamido derivatives; Tetrachlorophthalimido derivatives; Glycosylation

1. Introduction

Saponins are steroid or triterpenoid glycosides that are widely distributed in plants and in some marine organisms.[1,2] The carbohydrate residue, usually a mono-, di-, tri- or tetrasaccharide, is covalently attached to the sapogenin backbone. Examples include diosgenin, tigogenin or sarsapogenin. The carbohydrate chain constitutes a hydrophilic part, while the appropriate sapogenin is a hydrophobic fragment in this kind of glycoside. Usually, in natural diosgenyl glycosides β-D-glucopyranose is the first sugar attached to diogenin, which in turn has very often an α-L-rhamnopyranose substituted at the 2-position and another sugar or sugar chain at the 3- or 4-position.[1–9]

We have synthesized diosgenyl glycosides containing D-glucosamine derivatives as the carbohydrate residue. These glycosides have not been found thus far in natural sources. The presence of the D-glucosamine residue, particularly the NH_2 group, creates an opportunity to synthesize new derivatives of glycosides. We have reported general methods for the synthesis of this kind of diosgenyl glycosides. The most widely glycosylation methods are based on the participation of a group at C-2 of glycosyl donor, which favor formation of a 1,2-trans glycosidic bond. Also the leaving groups of the glycosyl donors have been recognized as one of the most important parameters responsible for the glycosylation reaction. Among the many methods of glycosylation now available, we decided to use the appropriate bromo and chloro sugars as glycosyl donors and silver triflate as the promoter.[10–12]

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Some of saponins exhibit a wide spectrum of biological activities, which can be ascribed to the diosgenin moiety and/or to the carbohydrate residue. Diosgenyl glycosides are often found as the major components in traditional Oriental medicines and have been used there as pharmaceutical agents to treat malaria, helminth infections and snake bites.[13–16] They are also good antifungal and antibacterial agents.[5,17] In the last two decades many pharmacological studies have shown that diosgenin and its glycosides exhibit cytotoxicity toward human cancer cells.[7,18–27] Dioscin,[4,6,28] polyphyllin D,[4,5] gracillin,[3] trillin[3] and saponin named Pb or PF3[15,20,29] are the most known diosgenyl saponins with these properties.

In a set of experiments we have investigated cells used from B cell chronic lymphocytic leukemia (B-CLL) patients, the most common form of leukemia in the Western world.[30] In this article we examined and compared the effects of the newly synthesized diosgenyl glycosides and 2-chlorodeoxyadenosine (2-CdA) on apoptosis B-CLL. Cladribine (2-CdA) is a purine analog recently introduced for the treatment of lymphoid malignancies.[31,32] The aims of the biological studies were to find out if the two diosgenyl glycosides and 2-CdA increase apoptosis and necrosis of B cells isolated from blood of B-CLL patients.

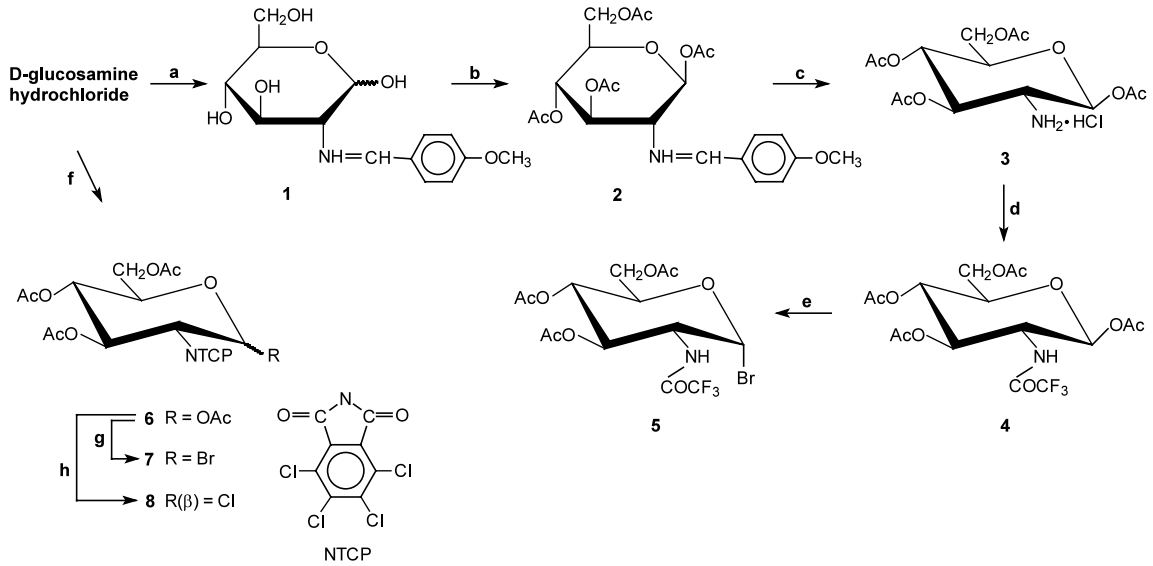
2. Results and discussion

2.1. Synthesis

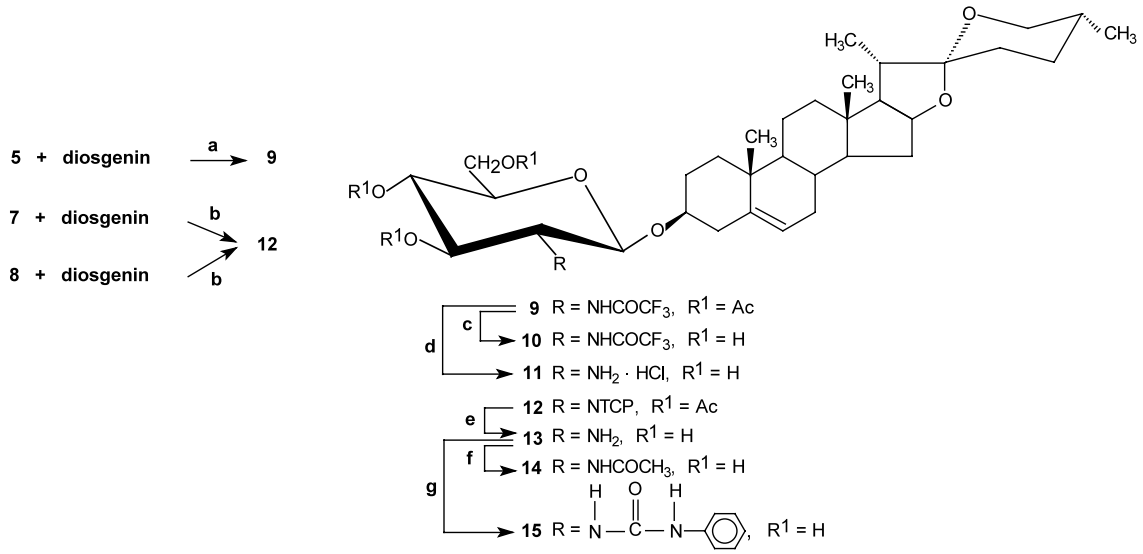
As demonstrated in the preceding paper,[33] the appropriate derivatives of D-glucosamine proved to be useful glycosyl donors for glycosylation of diosgenin. Among many methods the most widely used way involved a glycosyl donor containing a participating group as the amino-protecting function.[10] The Koenigs–Knorr reaction is one of the oldest method for the preparation of 1,2-trans-glycosides requiring 3,4,6-tri-*O*-acetyl-2-2-acylamido-2-deoxy-D-glucopyranosyl bromides and chlorides as donors and silver salts as promoters. In our route the synthetic strategy is based on preparation NTFAc- and NTCP-protected per-*O*-acetyl bromides and chlorides and subsequent coupling with diosgenin. The yield of the coupling reaction was usually moderate, probably due to the low reactivity of the glycosyl donors and the possibility of oxazoline derivative formation, as well as the reactivity of the acceptor.

Compound **3** was obtained from commercially available D-glucosamine hydrochloride in a procedure that used *p*-anisaldehyde for protection of NH_2 (→**1**), acetylation (→**2**) and removal of the *p*-methoxybenzylidene group with HCl in acetone (Scheme 1, route a–c).

This product was acylated again, but now with trifluoroacetic anhydride (→**4**), and finally **4** was treated with excess of $TiBr_4$ to give glycosyl donor **5** in good yield (80%). Application of trifluoroacetyl as a protecting group for the NH_2 function gave the possibility of selective deprotection of the hydroxyl and amino groups in the final glycoside. Subsequently, NTCP-derivative **6**, as a mixture of anomers, was obtained from D-GlcNH_2·HCl in a one-pot reaction sequence. First, the 3,4,5,6-tetrachlorophthalimido (TCP) group was installed by adding one equivalent of sodium methoxide in MeOH, followed by 3,4,5,6-tetrachlorophthalic anhydride. The NTCP-intermediate was



Scheme 1. Reagents and conditions: (a) aq NaOH, $CH_3O–C_6H_4–CHO$; (b) Py, Ac_2O; (c) acetone, 5 M HCl; (d) Py, $(CF_3CO)_2O$; (e) CH_2Cl_2 – EtOAc (10:1), $TiBr_4$; (f) NaOMe in MeOH, TCPO, Py, Ac_2O; (g) HBr–CH_3COOH; (h) $CHCl_3$, Cl_2HCOCH_3.



Scheme 2. Reagents and conditions: (a) CH_2Cl_2, AgOTf, -15 °C; (b) CH_2Cl_2, AgOTf, rt; (c) NaOMe in MeOH; (d) acetone, 0.1 M NaOH, 1.3% HCl in MeOH; (e) EtOH, $H_2NCH_2CH_2NH_2$ or EtOH, $NH_2NH_2{\cdot}H_2O$; (f) MeOH, Ac_2O; (g) C_6H_5NCO.

then O-acetylated with Ac_2O in pyridine which resulted in **6**. This procedure was adapted from Schmidt, who applied NTCP derivatives for the synthesis of series *O*-glycosides.[34,35] Treatment of **6** with excess of HBr in HOAc or with $TiBr_4$ resulted in the formation of glucosyl bromides **7** (white foam), also as an anomeric mixture.[36] Compound **6** was also transformed into the chloride **8** via the modified method (dichloromethyl + methyl ether and $BF_3{\cdot}Et_2O$) that has been used in the synthesis of the phthalimido derivatives in the galacto series.[37] Under these conditions only the crystalline anomer β was obtained (Scheme 1, route f–h).

Glycosylation of diosgenin with 3,4,6-tri-*O*-acetyl-2-deoxy-2-trifluoroacetamido-α-D-glucopyranosyl bromide (**5**) in the presence of AgOTf used in conjunction with activated and finally powdered 4Å molecular sieves in CH_2Cl_2 afforded exclusively β-glycoside **9** (Scheme 2, entry a, yield 69%). The 1,2-trans-linkage was ensured by the *N*-trifluoroacetyl neighboring-group participation.

Bromide **7** was also used as a glycosyl donor in the coupling reaction with diosgenin as an acceptor.[36] This reaction was carried out under almost the same conditions as those above and also gave the β glycoside **12** in 73% yield. The structures of these crystalline compounds have previously been determined in our laboratory by ^1H and ^{13}C NMR spectroscopy, together with X-ray analysis.[36]

In the next step, the coupling reaction of 3,4,6-tri-*O*-acetyl-2-deoxy-2-tetrachlorophthalimido-β-D-glucopyranosyl chloride (**8**) and diosgenin as an acceptor was carried out in the same manner as that for bromide **7** (Scheme 2, entry b). Here also the 1,2-*trans*-linkage was formed ($\rightarrow$**12**, 65%), because of the bulky NTCP group at carbon C-2, which is simultaneously serving as the neighboring participation group in the coupling reaction.

All three reactions were realized in the presence of an activator (AgOTf), which is called the reaction promoter, and in all of them only the β-glycosides have been obtained. The methods with use of glycosylation donors containing a participating group as the amino protective function in Koenigs–Knorr reactions are the so-called 'oxazoline' and 'phthalimido' procedures.[10] The role of AgOTf is to assist the departure of the anomeric leaving group (here Br or Cl) in a way giving intermediates that have a pronounced tendency to form the 1,2-*trans*-glycosides **9** and **12**.

The glycoside **9** was O-deacetylated by the Zemplén procedure[38] to give product **10** in high yield (98%). Finally, removal of all of the acyl groups on **9** with sodium hydroxide provided hydrochloride **11** (82%), (Scheme 2, entries c and d), which will be used as substrates for the synthesis of a new series of diosgenyl glycosides (e.g., *N*-ureido derivatives).

A TCP group in **12** could be rapidly and cleanly cleaved by use of a slight excess of ethylenediamine in ethanol.[34,35,39] Thus treatment of **12** with six equivalents of ethylenediamine in EtOH during 6 h at 60 °C gave fully deprotected glycoside **13** in 70% yield and *N*-acetyl-glycoside **14** in 29% yield, (Scheme 2, entries e and f). Alternatively, treatment of **12** with hydrazine hydrate results in only **13** (almost quantitatively). For biological purposes **13** was converted into hydrochloride **11**.

To confirm the structure of **14**, glycoside **13** was treated with acetic anhydride in methanol, which after workup in the usual manner gave the *N*-acetyl derivative **14**.[40]

Saponin **10** was also obtained in a good yield by *N*-acylation of the 2-amino sugar derivative **13** with trifluoroacetic anhydride in pyridine, followed by methanolysis.[41] Hydrochloride **11** (as a free base **13**) has been applied for the preparation of its *N*-aryl-ureido derivative. The most convenient method for the synthesis of ureido derivatives of amino sugars is based on the reaction of the appropriate isocyanate with the amino group.[42–44] Phenyl isocyanate was added to the solution of **13** (free base) in 1:1 chloroform–methanol, and after 30 minutes product **15** was isolated as a crystalline precipitate (Scheme 2, entry g). The structures of **9**–**15** were established on the basis of the ^{1}H and ^{13}C NMR spectroscopy.

2.2. Biological activity

A total of 27 patients with progressive or symptomatic B-CLL were enrolled in studies.[45] All patients were CD5+, CD19+ and CD23+ as were tested with monoclonal antibodies (DAKO, Denmark) with the use of a flow cytometry method (data not shown). Patients' B cells were cultivated 24 h alone or in the presence of **11** and **15** and 2-CdA. The B-cell apoptosis was detected by staining with FITC-labeled monoclonal antibodies against annexin V, which recognized phosphatidylserine on the surface apoptotic cells. During apoptosis the B-cell plasma membrane change included redistribution of phosphatidylserine from the cytoplasmatic surface to the outer leaflet making it available for annexin V binding. During later stages of apoptosis the plasma membranes become increasingly permeable and propidium iodide (PI) could move across the membrane and bind to DNA. Flow cytometry with annexin V/PJ double staining was used to distinguish necrotic and apoptotic B cells.[32] The effects of 24 h cells in incubation alone, with saponins **11**, **15**, and 2-CdA, and the mixture of **11** or **15** with 2-CdA on the amount of apoptotic (A) and necrotic (PI) cells are shown in Table 1. The spontaneous apoptosis and necrosis of leucemic B-cells (control) are indicated on very heterogeneous patient cell populations. The amount of apoptotic cells varies from a few percentage points (samples 7, 12) up to more than 50% (samples 22, 25). The incubation of leucemic B-cells with 2-CdA,

Table 1
The percentage of apoptotic (A) and necrotic (PI) leukemic B cell in the presence of Cladribine (2-CdA), **11** and **15**

n	Control		+2-CdA		+ 11		+2-CdA+11		+15		+2-CdA+15	
	A	PI	A	PI	A	PI	A	PI	A	PI	A	PI
1	42.6	23	23.4	9.2	24.4	8.5	19.1	7.7	20.1	7.2	16.5	5.7
2	29.9	30.8	49.4	10.5	48	9	46.7	3.1	45.5	8.9	46.9	4.1
3	12.7	34	12.1	37.6	12.9	38.8	11.7	36	14.7	13.4	13.1	23.2
4	31.4	27.1	29.7	7.8	23.3	7.2	30	7	25.1	7	41.9	10.8
5	24.3	21.5	20.5	15.3	21.5	18.9	21.1	16.2	21	18.6	19	19.1
6	5.2	1.4	4.4	0.8	5.3	1.4	7.2	0.8	5.4	1.1	6.3	1.1
7	7.2	3.7	10.2	6.3	10.7	7.3	10	6.5	11.9	6	10.9	7.9
8	12.3	6.1	9.8	4.3	10.9	5.3	9.4	6	10.6	6.3	10.2	6
9	18.3	1.3	32.9	2.2	15.3	0.9	37.5	1.4	10	0.2	35.5	2
10	44.3	20	55.8	12.7	54.1	14.3	56.7	14.1	53.3	15.8	–	–
11	18.1	8.8	21	3.2	13.2	3	17.8	3.5	12.3	2.6	15.2	2.6
12	4	1	3.8	0.9	4.2	0.9	9	5.8	3.6	0.8	5.7	2.4
13	37.5	11.7	29.4	4.9	28.7	5.1	24.1	2.2	28.3	5.4	17.1	1.1
14	35.7	46.4	47	33.3	47.2	32.9	45.4	27.9	43.6	33.5	52	21.4
15	2.3	0.5	2.5	0.3	2.9	0.3	3.3	0.4	2.1	0.2	3	0.2
16	12.2	1.5	6.3	0.4	7	0.8	5.4	0.6	5.3	0.4	6.2	0.4
17	12.3	4.3	11.4	1	9	1.3	10.1	0.7	6.6	0.9	10.7	0.4
18	14	15.3	8.1	2.9	7.7	3.4	11.4	23.6	7.5	2.2	6	3.8
19	30.2	34.9	26.2	23	25.9	23.4	20.9	27.8	26.9	24	22.7	20.8
20	21.3	9.2	22.4	9.2	18.6	13.6	16.1	8.3	18.1	6.1	15.3	8.8
21	15.7	11	19.6	8.1	16.4	10	24.4	7	17.2	5.1	22.9	10
22	52.5	21.4	62.5	14.8	61.3	12.2	54.9	10.4	59.8	10	59.5	15.2
23	37	15.9	33.7	17	40.9	8.8	19.2	4.6	24.5	1.4	28.7	6
24	23.1	14.5	27.2	6.4	14.9	3.2	27.9	7.4	13.2	3.6	31	6.6
25	51.1	31.7	59.4	19.7	60.5	23.6	50.7	28	53.2	24.2	57.5	13.4
26	17.1	7.7	24.5	14.9	12.9	2.7	15.1	4	13.8	3.3	17.1	6.2
27	28.8	12.9	27	5.5	27.8	6.1	17.8	2.2	27	6.3	15.6	2.9

Table 2
Evaluation of apoptosis (A) and necrosis (PI) of B cell isolated from B cell chronic lymphocytic leukemia patients

	+2-CdA		+11		+2-CdA+11		+15		+2-CdA+15	
	A	PI	A	PI	A	PI	A	PI	A	PI
no effect	11 (41) [a]	18 (67)	14 (52)	21 (78)	13 (48)	13 (48)	16 (59)	22 (81)	12 (44)	13 (48)
increase	11 (41)	4 (15)	6 (22)	2 (7)	3 (11)	9 (33)	6 (22)	0	6 (22)	5 (18)
decrease	5 (18)	5 (18)	7 (26)	4 (15)	11 (40)	5 (18)	5 (18)	5 (18)	9 (33)	9 (33)

[a] In brackets the percentage of total samples tested; 27 = 100%.

11 and **15** alone or with mixtures of the two shows no trend, as increases or decreases in apoptosis and necrosis depends on the samples tested. The results of Table 1 are summarized in Table 2. The effects of **11**, **15** and 2-CdA were estimated as significant if the amount of apoptotic cells decrease or increase for more then 5%, in comparison to each samples of untreated B cells (spontaneous A and PI). 2-CdA increases the amount of apoptotic cells in 11 (41%) and necrosis in 4 (15%) of the samples. The **11** and **15** were less effective than 2-CdA and increases apoptosis in 6 (22%) of the samples. Both saponins were also less effective on inducing the PI penetration, and only **11** induced it on 2 samples (7%). However, **11** and **15** enhanced the PI penetration in the presence of 2-CdA to 33% and 18%, respectively. It is worth stressing that **11** and **15** reduced the amount of annexin V binding samples, in comparison to 2-CdA alone. That may suggest changes on the outer surface membranes of tumor cells by both diosgenin derivatives. In summary, **11** and **15** are able to induce apoptosis and necrosis of some leukemic B-cells. The most important effect of **11** and **15** seems to be related with the increase of B-cell membrane permeability in some samples in the presence of 2-CdA. The testing of biological activities of **10** and **14** are planned. The comparison of all four diosgenyl derivatives may allow one to choose the most effective compound for consideration in antitumor therapy.

3. Experimental

3.1. General methods

Melting points were determined on a standard apparatus and were uncorrected. Optical rotations were measured on a Perkin–Elmer 343 polarimeter in a 1-dm cell at 20 °C. Thin-layer chromatography (TLC) was performed on aluminum sheets coated with Kieselgel 60 F_{254} (E. Merck, Darmstadt). The following solvent systems were used: A, 4:1 CCl_4–acetone; B, 5:1 toluene–ethyl acetate; C, 6:1 CCl_4–acetone; D, 2:1 toluene–ethyl acetate; E, 5:1 chloroform–methanol. Column chromatography was performed on Kieselgel 60 (< 0.08 mm; Macherey–Nagel). The organic extracts were dried over anhydrous Na_2SO_4, unless otherwise noted. For NMR measurements the samples were dissolved in the appropriate solvent, and Me_4Si was used as the internal reference. NMR spectra were recorded with a Varian Mercury spectrometer (400 MHz for ^1H and 100 MHz for ^{13}C). Elemental analyses were performed on a Carlo Erba EA1108 analyzer.

3.2. Preparation of 1,3,4,6-tetra-*O*-acetyl-β-D-glucosamine hydrochloride (3)

To a solution of D-glucosamine hydrochloride (25 g, 0.12 mol) in a freshly prepared aqueous solution of 1 M NaOH (120 mL) under stirring was added *p*-anisaldehyde (17 mL, 0.14 mol). After a short time crystallization began. Then the mixture was refrigerated and after 2 h the precipitated product was filtered off and washed with cold water, and followed by a mixture of 1:1 EtOH–Et_2O to give 2-deoxy-2-[*p*-methoxybenzylidene(amino)]-D-glucopyranose (**1**) (25.7 g, 72%): mp 165–166 °C, lit.[46] 163–164 °C. This intermediate product (25 g, 0.084 mol) was added successively to a cooled (ice-water) mixture of pyridine (135 mL) and Ac_2O (75 mL). The mixture was stirred in ice-bath ~ 1 h and then at room temperature overnight. The yellow solution was poured into 500 mL of ice-water. The precipitated white product was filtered, washed with cold water and dried. This product was identified as 1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-[*p*-methoxybenzylidene(amino)]-β-D-glucopyranose (**2**) (34.3 g, 88%): mp 180–182 °C, lit.[46] 188 °C; $[\alpha]_D^{20} + 95°$ (*c* 1.0, $CHCl_3$), lit.[46] + 98.6° ($CHCl_3$). Subsequently this compound (34 g, 0.073 mol) was dissolved in warm acetone (300 mL), and then HCl (5 M, 15 mL) was added with immediate formation of a precipitate. The mixture was cooled, and after addition of Et_2O (300 mL), it was stirred for 2 h and refrigerated overnight. The precipitated product was filtered, washed with Et_2O and dried to give **3** (24.9 g, 89%): at 235 °C this compound decomposed; $[\alpha]_D^{20} + 32°$ (*c* 1.0, MeOH), lit.[46] + 29.7° (H_2O).

3.3. Preparation of 1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-trifluoroacetamido-β-D-glucopyranose (4)

To a suspension of **3** (11.5 g, 30 mmol) in CH_2Cl_2 (100 mL) and pyridine (12 mL), under cooling (ice-water) and stirring, trifluoroacetic anhydride (12 mL) was added. After stirring for 0.5 h the solution was poured into ice-water. The organic phase was washed with water (100 mL), dried and concentrated. Addition of Et_2O yielded crystals of **4** (12.1 g, 91%): mp 164–165 °C, lit.[47]: mp 167 °C; $[\alpha]_D^{20}$ −10° (*c* 1.0, $CHCl_3$), lit.[47] −13° (*c* 2.4, $CHCl_3$).

3.4. Preparation of 3,4,6-tri-*O*-acetyl-2-deoxy-2-trifluoroacetamido-α-D-glucopyranosyl bromide (5)

To a solution of **4** (6.65 g, 15 mmol) in dry CH_2Cl_2 (150 mL) and dry EtOAc (15 mL) was added $TiBr_4$ (6 g, 16 mmol). The mixture was stirred at room temperature and monitored by TLC (solvent A). After 0.5 h a second portion of $TiBr_4$ (6 g, 16 mmol) was added, and after stirring for 4 h the red solution was diluted with $CHCl_3$ (350 mL) and washed with water (2 × 150 mL). The organic layer was dried and filtered, and the solvents were evaporated. The residue was crystallized from Et_2O–hexane to yield bromide **5** (5.5 g, 80%): mp 92–94 °C, lit.[47] mp 96 °C; $[\alpha]_D^{20}$ +122° (*c* 1.0, $CHCl_3$), lit.[47] +126° (*c* 2.92, $CHCl_3$).

3.5. 1,3,4,6-Tetra-*O*-acetyl-2-deoxy-2-(3,4,5,6-tetrachlorophthalimido)-α,β-D-glucopyranose (6)

D-Glucosamine hydrochloride (2.16 g, 0.01 mol) was added to a NaOMe solution prepared from Na (0.23 g, 0.01 mol) and MeOH (10 mL). After 15 min the mixture was treated with 3,4,5,6-tetrachlorophthalic anhydride (1.43 g, 5 mmol) and shaken for 20 min. Afterwards Et_3N (1.4 mL, 0.01 mol) was added, and the reaction mixture was treated again with the anhydride (1.43 g, 5 mmol). The mixture was then warmed at 50 °C and stirred for 1 h. After concentration, the residue was treated with pyridine (20 mL) and Ac_2O (20 mL) at room temperature for 24 h. The solution was poured into ice-water, and the aqueous mixture was extracted with $CHCl_3$ (3 × 100 mL). The organic extracts were combined, successively washed with H_2O, HCl (3%), satd aq $NaHCO_3$ and H_2O. The organic layer was dried and filtered, and the solvent was evaporated. Addition of MeOH caused precipitation of **6** as a white solid (2.7 g, 44%). An additional portion of **6** (1.1 g, 18%) was obtained after column chromatography (system B). ^1H NMR ($CDCl_3$) for **6**: δ 1.9–2.21 (4s, 12 H, 4 OAc), 4.25 (dd, 1 H, $J_{5,6}$ 4.8 Hz, H-6β), 4.35 (dd, 1 H, H-6′β), 4.28–4.4 (m, 2 H, H-5α, H-6α), 4.45 (dd, 1 H, $J_{1,2}$ 8.8 Hz, $J_{2,3}$ 10 Hz, H-2β), 4.71 (dd, 1 H, $J_{1,2}$ 3.6 Hz, $J_{2,3}$ 11.6 Hz, H-2α), 5.23 (dd, 1 H, $J_{3,4}$ 9.2 Hz, $J_{4,5}$ 10 Hz, H-4β), 5.68 (dd, 1 H, $J_{2,3}$ 10 Hz, $J_{3,4}$ 9.2 Hz, H-3β), 6.26 (d, 1 H, $J_{1,2}$ 3.6 Hz, H-1α), 6.46 (d, 1 H, $J_{1,2}$ 8.8 Hz, H-1β), 6.48 (dd, 1 H, $J_{2,3}$ 11.6 Hz, $J_{3,4}$ 9.2 Hz, H-3α), 5.16 (dd, 1 H, $J_{3,4}$ 9.2 Hz, $J_{4,5}$ 10.4 Hz, H-4α). Anal. Calcd for $C_{22}H_{19}Cl_4NO_{11}$: C, 42.95; H, 3.11; N, 2.28. Found: C, 43.02; H, 3.10; N, 2.28.

3.6. 3,4,6-Tri-*O*-acetyl-2-deoxy-2-(3,4,5,6-tetrachlorophthalimido)-β-D-glucopyranosyl chloride (8)

To a solution of **6** (0.3 g, 0.5 mmol) in dry $CHCl_3$ (2 mL) Cl_2HCOCH_3 (0.55 mL, 4.4 mmol) and $BF_3{\cdot}Et_2O$ (0.3 mL) were added. After being stirred for 10 min under N_2, the reaction mixture was heated for 48 h at 60 °C. Then it was diluted with $CHCl_3$ (20 mL), washed with H_2O and satd aq $NaHCO_3$, dried over anhydrous $MgSO_4$, and concentrated to give a foam, which was crystallized from Et_2O–petroleum ether to afford **8** (0.2 g, 67%): mp 154–156 °C; $[\alpha]_D^{20}$ + 70° (*c* 0.5, $CHCl_3$); R_f 0.57 (system C); ^1H NMR ($CDCl_3$): δ 1.92 (s, 3 H, OAc), 2.05 (s, 3 H, OAc), 2.14 (s, 3 H, OAc), 3.94 (m, 1 H, $J_{4,5}$ 10.26 Hz, H-5), 4.21 (dd, 1 H, $J_{5,6'}$ 2.2 Hz, H-6′), 4.34 (dd, 1 H, $J_{5,6}$ 4.8 Hz, H-6), 4.51 (dd, 1 H, $J_{2,3}$ 10.26 Hz, $J_{3,4}$ 9.15 Hz, H-2), 5.27 (dd, 1 H, $J_{2,3}$ 10.26 Hz, $J_{3,4}$ 9.15 Hz, H-3), 6.7 (d, 1 H, $J_{1,2}$ 9.2 Hz, H-1). Anal. Calcd for $C_{20}H_{16}Cl_5NO_9$: C, 40.60; H, 2.73; N, 2.37. Found: C, 40.79; H, 2.66; N, 2.31.

3.7. Diosgenyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-trifluoroacetamido-β-D-glucopyranoside (9)

To a cooled (ca. −15 °C) solution of diosgenin (0.42 g, 1 mmol) in anhyd CH_2Cl_2 (100 mL) with stirring under N_2 type 4 Å crushed molecular sieves (6 g) and bromide **5** (0.93 g, 2 mmol) were successively added. AgOTf (0.77 g, 3 mmol) dissolved in anhyd Et_2O (25 mL) was then added dropwise during 50 min. This mixture was stirred under N_2 at ca. −15 °C for 30 min. After this time TLC (solvent A and D) showed the conversion of substrate into products. The mixture was filtered and evaporated to dryness. The syrup was chromatographed (solvent B) to remove unreacted diosgenin and to purify the coupling product. It afforded pure **9** (0.55 g, powder, 69%): mp >220 °C (dec); $[\alpha]_D^{20}$ −74° (*c* 0.6, MeOH); ^1H NMR data see ref.[33]; ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.73 (C-21), 16.48 (C-18), 17.34 (C-27), 19.51 (C-19), 20.68 ($CO\mathit{C}H_3$), 20.81 ($CO\mathit{C}H_3$), 20.97 ($CO\mathit{C}H_3$), 21.03 (C-11), 28.98, 29.58, 30.49, 31.57 (C-8, C-24, C-25, C-23, C-2), 32.03 (C-15), 32.26 (C-7), 37.03 (C-10), 37.31 (C-1), 38.78 (C-4), 39.93 (C-12), 40.46 (C-13), 41.80 (C-20), 50.26 (C-9), 55.50 (C-2′), 56.68 (C-14), 62.28 (C-6′, C-17), 67.05 (C-26), 68.70 (C-4′), 71.83 (C-3′), 72.00 (C-5′), 80.06 (C-3), 80.99 (C-16), 99.01 (C-1′), 109.51 (C-22), 122.23 (C-6), 140.32 (C-5), 169.55, 170.93, 171.27 ($\mathit{C}OCH_3$ × 3, $\mathit{C}OCF_3$). Anal. Calcd for $C_{41}H_{58}F_3NO_{11}$: C, 61.72; H, 7.33; N, 1.76. Found: C, 61.72; H, 7.40; N, 1.65.

3.8. Diosgenyl 2-deoxy-2-trifluoroacetamido-β-D-glucopyranoside (10)

To a solution of **9** (0.32 g, 0.4 mmol) in MeOH (100 mL) a 1 M solution of NaOMe in MeOH (5 mL) was added. The mixture was stirred at room temperature for 0.5 h and was then neutralized with Dowex-50W (H^+) ion-exchange resin (pH was adjusted to 7; pH meter) and filtered, and the solvent was evaporated. The semisolid residue was dissolved 1:1 in $CHCl_3$–MeOH and filtered and again concentrated to dryness to give **10** (0.26 g, powder, 98%): mp >217 °C (dec.); $[\alpha]_D^{20}$ $-53°$ (*c* 0.8, MeOH); ^1H NMR ($(CD_3)_2SO$): δ 3.1 (m, 2 H, H-3, H-5), 3.4 (m, 3 H, H-2, H-4, H-6′), 3.75 (dd, 1 H, H-6), 4.45–4.5 (m, 1 H, 6-OH), 4.5 (d, 1 H, $J_{1,2}$ 9.2 Hz, H-1), 5.05 (m, 1 H, 3-OH), 5.15 (m, 1 H, 4-OH), 9.15 (d, 1 H, *J* 8.4 Hz, NH); diosgenyl protons: 0.72 (d, CH_3), 0.76 (s, CH_3), 0.9 (d, CH_3), 0.92 (s, CH_3), 1.9 (m, $C_{(15)}$-H), 2.28 (m, $C_{(15)}$-H′), 4.28 (dd, $C_{(3)}$–H), 5.3 (m, $C_{(6)}$–H). ^{13}C NMR (100 MHz, $(CD_3)_2SO$): δ 14.56 (C-21), 15.91 (C-18), 16.99 (C-27), 18.96 (C-19), 20.30 (C-11), 28.42, 29.10, 29.74, 30.90, 31.40, 31.48 (C-8, C-24, C-25, C-23, C-2, C-7, C-15), 36.28 (C-10), 36.64 (C-1), 38.25 (C-4), 38.88 (C-12), 40.13 (C-13), 41.03 (C-20), 49.47 (C-9), 55.67 (C-14), 56.36 (C-2′), 60.87 (C-6′), 61.75 (C-17), 65.86 (C-26), 70.44 (C-5′), 73.10 (C-4′), 77.02 (C-3′), 77.53 (C-3), 80.13 (C-16), 98.53 (C-1′), 108.35 (C-22), 121.14 (C-6), 140.23 (C-5), 158.00 ($COCF_3$). Anal. Calcd for $C_{35}H_{52}F_3NO_8 \cdot 1.5H_2O$: C, 60.15; H, 7.93; N, 2.00. Found: C, 60.24; H, 7.63; N, 1.97.

Compound **10** was also obtained from **13** in the following procedure: to 0.1 g of **13** dissolved in dry pyridine (2 mL) and cooled in ice, 0.3 mL trifluoroacetic anhydride in dry Et_2O (2 mL) was added dropwise. After 24 h, the solution was diluted with EtOAc, washed with H_2O, dried ($MgSO_4$) and after concentration purified by silica gel-column chromatography (10:1 $CHCl_3$–MeOH) to afford **10** as a white solid (0.085 g, 60%).

3.9. Diosgenyl 2-amino-2-deoxy-β-D-glucopyranoside hydrochloride (11)

The coupled product **9** (0.33 g, 0.4 mmol) was dissolved in acetone (65 mL) and then a 0.1 M solution of NaOH (32 mL) was added. After stirring for 2 h at room temperature, the pH of the mixture was adjusted to 7 (pH meter) by adding Dowex-50W ion-exchange resin, and then the mixture was filtered. Concentration of the filtrate yielded a syrup, which was dissolved in EtOH and concentrated again. The semisolid residue was dissolved in 1:1 $CHCl_3$–MeOH and filtered, and a stoichiometric amount of 1.3% HCl in MeOH was added. The hydrochloride was precipitated with petroleum ether to give **11** as an amorphous powder (0.21 g, 82%): $[\alpha]_D^{20}$ $-56°$ (*c* 0.4, 1:1 $CHCl_3$–MeOH); ^1H NMR data see ref.[33]; ^{13}C NMR (100 MHz, $CDCl_3$–CD_3OD): δ 15.00 (C-21), 16.94 (C-18), 17.62 (C-27), 19.99 (C-19), 21.90 (C-11), 29.18, 30.64, 31.25, 32.31, 32.59, 32.65, 33.07 (C-8, C-24, C-25, C-23, C-2, C-7, C-15), 37.86 (C-10), 38.29 (C-1), 39.71 (C-4), 40.76 (C-12), 41.29 (C-13), 42.72 (C-20), 51.33 (C-9), 57.56 (C-2′), 57.95 (C-14), 62.61 (C-6′), 63.29 (C-17), 67.74 (C-26), 71.60 (C-4′), 77.10, 77.62 (C-3′, C-5′), 79.58 (C-3), 81.96 (C-16), 102.11 (C-1′), 110.38 (C-22), 122.47 (C-6), 141.25 (C-5). 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.

3.10. Diosgenyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-(3,4,5,6-tetrachlorophthalimido)-β-D-glucopyranoside (12)

To a mixture of **8** (0.87 g, 1.4 mmol), diosgenin (0.4 g, 1 mmol) and 4 Å molecular sieves (4 g) in anhyd CH_2Cl_2 (68 mL) at room temperature under N_2, AgOTf (0.75 g, 2.92 mmol) in dry Et_2O (45 mL) was added. After stirring 1 h, the mixture was neutralized by Et_3N (2 mL), diluted with $CHCl_3$, filtered and concentrated. Addition of MeOH caused precipitation of **12** as a white solid (0.62 g, 65%); mp 257–258 °C, $[\alpha]_D^{20}$ $+12°$ (*c* 0.45, $CHCl_3$); for ^1H, ^{13}C NMR and X-ray crystallography data see ref.[36].

3.11. Diosgenyl 2-amino-2-deoxy-β-D-glucopyranoside (13) and diosgenyl 2-acetamido-2-deoxy-β-D-glucopyranoside (14)

Compound **12** (0.2 g, 0.21 mmol) was dissolved in EtOH (4 mL), and then ethylenediamine (0.074 g, 1.23 mmol) was added. The reaction mixture was heated at 60 °C for 6 h and then concentrated. The residue was applied to a silica gel column (5:1 $CHCl_3$–MeOH, containing 0.2% Et_3N) to give **13** as a white solid (0.083 g, 70%) and **14** (0.037 g, 29%). For **13**: mp 230 °C (dec.); $[\alpha]_D^{20}$ $-102°$ (*c* 0.5, 1:1 $CHCl_3$–CH_3OH); ^1H NMR (CD_3OD): δ 2.62 (dd, 1 H, $J_{1,2}$ 8.1 Hz, $J_{2,3}$ 9.3 Hz, H-2), 3.26 (m, 1 H, $J_{4,5}$ 8.3 Hz, $J_{5,6}$ 5 Hz, H-5), 3.30 (m, 1 H, $J_{2,3}$ 9.3 Hz, $J_{3,4}$ 8.3 Hz, H-3), 3.33 (dd, 1 H, $J_{3,4}$ 8.3 Hz, $J_{4,5}$ 8.3 Hz, H-4), 3.71 (dd, 1 H, $J_{5,6}$ 9 Hz, H-6), 3.84 (dd, 1 H, $J_{5,6'}$ 2.3 Hz, H-6′), 4.37 (d, 1 H, $J_{1,2}$ 8.1 Hz, H-1); diosgenyl protons: 0.72 (s, CH_3), 0.76 (s, CH_3), 0.9 (d, CH_3), 0.92 (s, CH_3), 1.9 (m, $C_{(15)}$–H), 2.28 (m, $C_{(15)}$–H′), 3.32 (m, $C_{(26)}$–$H_{(a)}$), 3.45 (m, $C_{(26)}$–$H_{(e)}$), 3.58 (m, $C_{(16)}$–H), 4.4 (dd, $C_{(3)}$–H), 5.35 (m, $C_{(6)}$–H); ^{13}C NMR (100 MHz, $CDCl_3$–CD_3OD): δ 14.97 (C-21), 16.90 (C-18), 17.59 (C-27), 19.96 (C-19), 21.81 (C-11), 29.61, 30.56, 31.16, 32.22, 32.47, 32.57, 32.98 (C-8, C-24, C-25, C-23, C-2, C-7, C-15), 37.77 (C-10), 38.19 (C-1), 39.65 (C-4), 40.66 (C-12), 41.21 (C-13), 42.62 (C-20), 51.20 (C-9), 57.46 (C-2′), 57.83 (C-14), 62.57 (C-6′), 63.13 (C-17), 67.67 (C-26), 71.51 (C-4′), 77.07,

77.43 (C-3′, C-5′), 79.52 (C-3), 81.88 (C-16), 102.14 (C-1′), 110.33 (C-22), 122.41 (C-6), 141.13 (C-5). For **14**: mp >238 °C (dec.); $[\alpha]_D^{20} -75°$ (*c* 0.5, 1:1 $CHCl_3$–CH_3OH); ^1H NMR ($(CD_3)_2SO$): δ 3.05 (m, 2 H, H-3, H-5), 3.3–3.4 (m, 3 H, H-2, H-4, H-6′), 3.66 (dd, 1 H, $J_{5,6}$ 5.6 Hz, H-6), 4.4 (d, 1 H, $J_{1,2}$ 9.2 Hz, H-1), 4.35–4.45 (m, 1 H, 6-OH), 4.82 (m, 1 H, 4-OH), 4.89 (m, 1 H, 3-OH), 7.61 (d, 1 H, *J* 8.4 Hz, NH); diosgenyl protons: 0.73 (d, CH_3), 0.74 (s, CH_3), 0.9 (d, CH_3), 0.95 (s, CH_3), 1.9 (m, $C_{(15)}$–H), 2.3 (m, $C_{(15)}$–H′), 4.28 (dd, $C_{(3)}$–H), 5.31 (m, $C_{(6)}$–H); ^{13}C NMR (100 MHz, $(CD_3)_2SO$): δ 14.57 (C-21), 15.92 (C-18), 17.00 (C-27), 19.06 (C-19), 20.32 (C-11), 23.03 ($CO\mathit{C}H_3$), 28.42, 29.17, 29.74, 30.88, 30.93, 31.41, 31.49 (C-8, C-24, C-25, C-23, C-2, C-7, C-15), 36.00 (C-10), 36.74 (C-1), 38.43 (C-4), 38.88 (C-12), 40.13 (C-13), 41.04 (C-20), 49.51 (C-9), 55.69 (C-14), 55.83 (C-2′), 61.06 (C-6′), 61.76 (C-17), 65.87 (C-26), 70.67 (C-5′), 74.19 (C-4′), 76.88 (C-3′), 77.41 (C-3), 80.13 (C-16), 99.33 (C-1′), 108.35 (C-22), 120.99 (C-6), 140.46 (C-5), 168.90 ($\mathit{C}OCH_3$).

When **13** (0.025 g, 0.0434 mmol) in MeOH (6 mL) was treated with Ac_2O (1.2 mL) and Et_3N (0.12 mL), it gave 0.02 g of **14**, identical with the sample prepared during deprotection of **12**.

3.12. Diosgenyl 2-amino-2-deoxy-β-D-glucopyranoside (13) from the reaction of 12 with hydrazine hydrate

Glycoside **12** (0.2 g, 0.206 mmol) in EtOH (4 mL) was heated with hydrazine hydrate (0.35 mL of an 85% solution) for 24 h. After this time the solvent was evaporated, and the residue was dried under high vacuum to remove traces of hydrazine. Silica gel column chromatography (solvent E) of this residue afforded **13** (0.12 g, 97%), which was isolated as the hydrochloride **11**, after adding to the solution stoichiometric amount of 1.3% HCl in MeOH.

3.13. Diosgenyl 2-deoxy-2-phenylureido-β-D-glucopyranoside (15)

To a solution of **11** (0.15 g, 0.25 mmol) in 1:1 $CHCl_3$–MeOH (30 mL) under stirring was added Dowex 2X-8 ion-exchange resin (HCO_3^-) to obtain **13** (the free base of **11**). After a short time (ca. 10 min), the mixture was filtered, and phenyl isocyanate (86 μL, 0.8 mmol) was added. It was stirred for 1 h at room temperature until TLC (solvent E) showed the complete conversion of **13** into the product. Then the solvents were evaporated to dryness. The semisolid residue was dissolved in 1:1 chloroform–methanol, and the mixture was filtered and concentrated to a low volume. Addition of hexane afforded the product as a white solid, which was separated on a centrifuge, washed with hexane and centrifuged again to give **15** as an amorphous powder (0.14 g, 80%): $[\alpha]_D^{20} -11°$ (*c* 0.4, 1:1 $CHCl_3$–CH_3OH); ^1H NMR (CD_3OD): δ 3.26–3.34 (m, 4 H, H-2, H-3, H-4, H-5), 3.68 (dd, 1 H, $J_{5,6}$ 5.2 Hz, H-6), 3.88 (dd, 1 H, $J_{5,6'}$ 2.0 Hz, H-6′), 4.60 (d, 1 H, $J_{1,2}$ 8.4 Hz, H-1), 6.98 (t, 1 H, Ph), 7.25 (t, 2 H, Ph), 7.35 (d, 2 H, Ph), 7.41 (d, 1 H, *J* 8.8 Hz, NH); diosgenyl protons: 0.78 (s, CH_3), 0.80 (s, CH_3), 0.96 (d, CH_3), 0.99 (s, CH_3), 2.20 (m, $C_{(15)}$–H), 2.39 (m, $C_{(15)}$–H′), 3.39 (m, $C_{(26)}$–$H_{(a)}$), 3.44 (m, $C_{(26)}$–$H_{(e)}$), 3.54 (m, $C_{(16)}$–H), 4.39 (dd, $C_{(3)}$–H), 5.32 (m, $C_{(6)}$–H). Anal. Calcd for $C_{40}H_{58}N_2O_8 \cdot 1.5H_2O$: C, 66.55; H, 8.52; N, 3.88. Found: C, 66.21; H, 8.19; N, 4.01.

3.14. Biological methods

3.14.1. Cell separation. Periferial blood mononuclear cells (PBMC) were collected into sterile, versanian-containing tubes. The PMBC were separated by differential centrifugation on a Ficoll-Hypaque centrifuge (Lymphoprep; Nyegard, Oslo, Norway).

3.14.2. Culture conditions. B-CLL separated cells were cultured (2×10^6 cells/mL) in complete medium (RPMI 1640, Bio-Whittaker, Walkersville, MD, USA), supplemented with 10% heat-inactivated FCS with or without 2-chlorodeoxyadenosidne (Leustain; Ortho Biotech, Raritan, NJ, USA), **11** and **15** for 24 h, at final concentration 1.75×10^{-7} M (50 ng/mL) in a fully humidified atmosphere of 5% CO_2 at 37 °C. In some experiments **11** or **15** were added simultaneously with 2-CdA, each at a concentration 1.75×10^{-7} M (50 ng/mL).

3.14.3. Analysis of apoptosis. The cells were centrifuged, washed in PBS and stained with Annexin V and propidium iodide according the manufacturer's protocol (BioSource International, Inc., Camarillo, CA, USA).

The distribution of cellular DNA content and apoptotic cells were determined after staining with propidium iodide and Annexin V by flow cytometry using a Epics XL Coulter flow cytometer with argon laser excitation at 488 nm.

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References

1. Mahato, S. B.; Ganguly, A. N.; Sahu, N. P. *Phytochemistry* **1982**, *21*, 959–978.
2. Bersuker, I. B.; Dimoglo, A. S.; Choban, I. N.; Lazurewskii, G. V.; Kintya, P. K. *Khim.-Farm. Zh*. **1983**, *17*, 1467–1471.
3. Li, C.; Yu, B.; Liu, M.; Hui, Y. *Carbohydr. Res*. **1998**, *306*, 189–195.
4. Deng, S.; Yu, B.; Hui, Y.; Yu, H.; Han, X. *Carbohydr. Res*. **1999**, *317*, 53–62.
5. Li, B.; Yu, B.; Hui, Y.; Li, M.; Han, X.; Fung, K.-P. *Carbohydr. Res*. **2001**, *331*, 1–7.
6. Yu, B.; Tao, H. *Tetrahedron Lett*. **2001**, *42*, 2405–2407.
7. Ikeda, T.; Miyashita, H.; Kajimoto, T.; Nohara, T. *Tetrahedron Lett*. **2001**, *42*, 2353–2356.
8. Yang, F.; Du, Y. *Carbohydr. Res*. **2002**, *337*, 485–491.
9. Han, X. W.; Yu, H.; Liu, X. M.; Bao, X.; Yu, B.; Li, C.; Hui, Y. Z. *Magn. Reson. Chem*. **1999**, *37*, 140–144.
10. Banoub, J.; Boullanger, P.; Lafont, D. *Chem. Rev*. **1992**, *92*, 1167–1195.
11. Schmidt, R. R.; Kinzy, W. *Adv. Carbohydr. Chem. Biochem*. **1994**, *50*, 21–123.
12. Bednarczyk, D.; Myszka, H. *Wiad. Chem*. **2001**, *55*, 491–515.
13. Khanna, I.; Seshadri, R.; Seshadri, T. R. *Indian J. Chem*. **1975**, *13*, 781–784.
14. Espejo, O.; Llavot, J. C.; Jung, H.; Giral, F. *Phytochemistry* **1982**, *21*, 413–416.
15. Miyamura, M.; Nakano, K.; Nohara, T.; Tomimatsu, T.; Kawasaki, T. *Chem. Pharm. Bull*. **1982**, *30*, 712–718.
16. Ma, J. C. N.; Lau, F. W. *Phytochemistry* **1985**, *24*, 1561–1565.
17. Hufford, C. D.; Liu, S.; Clark, M. A. *J. Nat. Prod*. **1988**, *51*, 94–98.
18. Ravikumar, P. R.; Hammesfahr, P.; Sigh, Ch. J. *J. Pharm. Sci*. **1979**, *68*, 900–903.
19. Lay, J. Y.; Chiang, H. C. *J. Taiwan Pharmaceut. Assoc*. **1980**, *32*, 14–28.
20. Koseki, Y.; Swetman, T. W.; Israel, M.; Hermann, J.; Potmesil, M.; Liu, L. F. *J. Cancer Res. Clin. Oncol*. **1990**, *116* (Suppl. 1), 620.
21. Wu, R. T.; Chiang, W. C.; Fu, W. C.; Chien, K. Y.; Chung, Y. M.; Horng, L. Y. *Int. J. Immunopharmacol*. **1990**, *12*, 777–786.
22. Takechi, M.; Tanaka, Y. *Phytochemistry* **1991**, *30*, 2557–2558.
23. Pettit, G. R.; Doubek, D. L.; Herald, D. L. *J. Nat. Prod*. **1991**, *54*, 1491–1502.
24. Nappez, C.; Liagre, B.; Beneytout, J. L. *Cancer Lett*. **1995**, *96*, 133–140.
25. Beneytout, J. L.; Nappez, C.; Leboutet, M. J.; Malinvaud, G. *Biochem. Biophys. Res. Commun*. **1995**, *207*, 398–404.
26. Ma, X.; Yu, B.; Hui, Y.; Xiao, D.; Ding, J. *Carbohydr. Res*. **2000**, *329*, 495–505.
27. Moalic, S.; Liagre, B.; Corbiere, C.; Bianchi, A.; Dauca, M.; Bordji, K.; Beneytout, J. L. *FEBS Lett*. **2001**, *506*, 225–230.
28. Deng, S.; Yu, B.; Hui, Y. *Tetrahedron. Lett*. **1998**, *39*, 6511–6514.
29. Kosuge, Jpn. Patent, Kokai Tokkyo Koho, JP 60 01,130, 1985; IntCl. A61K31/705//C07J71/00; *Chem. Abstr*., **1985**, *102*, 191170n.
30. Cheson, B. C. *J. Clin. Oncol*. **1992**, *10*, 352–355.
31. Robak, T.; Błoński, J. Z.; Kasznicki, M.; Konopka, L.; Ceglarek, B.; Domoszyńska, A.; SorokaWojtaszko, M.; Skotnicki, A. B.; Nowak, W.; Dwilewicz-Trojaczek, J.; Tomaszewska, A.; Hellman, A.; Lewandowski, K.; Kuliczkowski, K.; Potoczedk, S.; Zdziarska, B.; Hansz, J.; Kroll, R.; Komarnicki, M.; Hołowiecki, J.; Grieb, P. *British J. Haematol*. **2000**, *108*, 368–375.
32. Castejon, R.; Vargas, J. A.; Briz, M.; Romero, Y.; Gea-Bancloche, J. C.; Fernandez, M. N.; Dutrantez, A. *Leukemia* **1997**, *11*, 1253–1257.
33. Bednarczyk, D.; Kaca, W.; Myszka, H.; Serwecińska, L.; Smiatacz, Z.; Zaborowski, A. *Carbohydr. Res*. **2000**, *328*, 249–252.
34. Castro-Palomino, J. C.; Schmidt, R. R. *Tetrahedron Lett*. **1995**, *36*, 5343–5346.
35. Castro-Palomino, J. C.; Schmidt, R. R. *Justus Liebigs Ann. Chem*. **1996**, 1623–1626.
36. Bednarczyk, D.; Myszka, H.; Ciunik, Z.; Kaca, W. *Magn. Reson. Chem*. **2002**, *40*, 231–236.
37. Nilsson, U.; Ray, A. K.; Magnusson, G. *Carbohydr. Res*. **1990**, *208*, 260–263.
38. Zemplen, G.; Gerecs, A. *Ber. Dtsch. Chem. Ges*. **1934**, *67*, 2049–2051.
39. Griffith, S. L.; Madsen, R.; Fraser-Reid, B. *J. Org. Chem*. **1967**, *62*, 3654–3658.
40. Bundle, D. R.; Josephson, S. *J. Chem. Soc., Perkin Trans. 1* **1981**, 2736–2739.
41. Israel, M.; Murray, M. J. *J. Med. Chem*. **1982**, *25*, 24–28.
42. Johnston, T. P.; McCaleb, G. C.; Opliger, P. S.; Laster, W. R.; Montgomery, J. A. *J. Med. Chem*. **1971**, *14*, 600–614.
43. Suami, T.; Tadano, K. *J. Med. Chem*. **1979**, *22*, 314–316.
44. Weinkam, R. J.; Lin, H. S. *Adv. Pharmacol. Chemother*. **1982**, *19*, 1–33.
45. Matutes, E.; Owusu-Ankomah, K.; Morilla, R.; Marco, J. G.; Houlihan, A.; Que, T. H.; Catovsky, D. *Leukemia* **1994**, *8*, 1640–1645.
46. Bergmann, M.; Zervas, L. *Ber. Dtsch. Chem. Ges*. **1931**, *64*, 975–980.
47. Wolfrom, M. L.; Bhat, H. B. *J. Org. Chem*. **1967**, *32*, 1821–1823.