ORGANIC LETTERS

2005
Vol. 7, No. 4
669−672

Fluorophore-Appended Steroidal Saponin (Dioscin and Polyphyllin D) Derivatives

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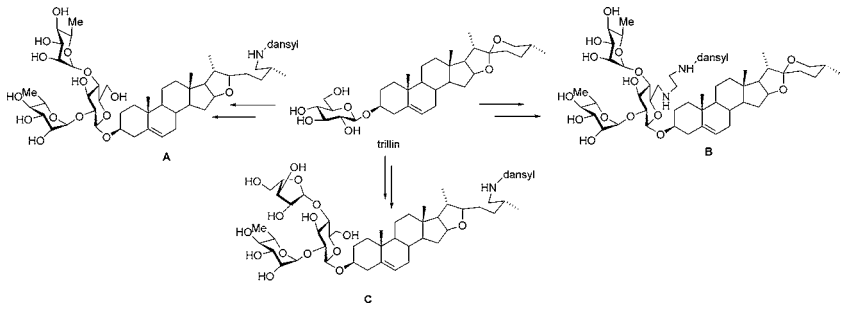
Received November 28, 2004

ABSTRACT



The synthesis of three fluorophore-appended derivatives of dioscin and polyphyllin D is reported herein. Starting from trillin, dansyl derivatives A–C were prepared in overall yields of 7–12% over 7–10 steps. A study of their behavior in a variety of polar solvents suggests that dansyl derivatives A–C are capable of micellar self-assembly and can maintain cytotoxicities (IC_{50} = 15–18 μM) against the HeLa carcinoma cell line evaluated by standard MTT assay.

Saponins, a class of glycoconjugates, are widely found in terrestrial plants and some marine organisms and are reported to be active constituents of many well-known traditional chinese medicines.[1] Structurally, saponins contain two distinct components, a saccharide (or polysaccharide) group and a sapogenin moiety, with the latter being usually a steroid or triterpene unit. Both components are important to the bioactivities exhibited by saponins.[2] The molecular structures of two typical steroidal saponins, dioscin (diosgenyl 2,4-di-*O*-α-L-rhamnopyrano-syl-*β*-D-glucopyranoside) and polyphyllin D (diosgenyl α-L-rhamno-pyranosyl-(1→2)-[α-L-arabinofuranosyl-(1→4)]-*β*-D-glucopyranoside), are depicted in Figure 1. Dioscin exhibits moderate to good cardiovascular, cytotoxic, and antifungal activities.[3,4] Polyphyllin D

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10.1021/ol0475616 CCC: $30.25
Published on Web 01/27/2005

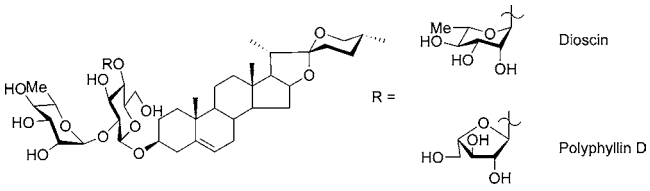
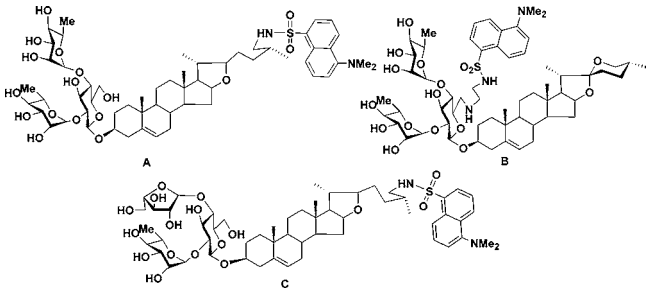


Figure 1. Structures of dioscin and polyphyllin D.

has been shown to have promising cardiovascular, antitumor, hemostatic, and immunomodulating effects.[5] The synthesis of these two saponins has been reported previously.[6]

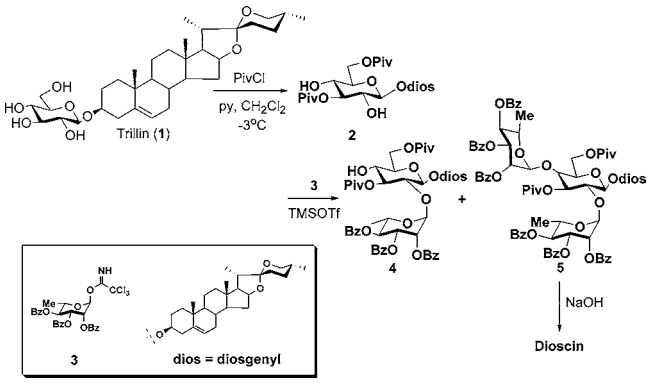
To examine the pharmacological mechanisms of these two diosgenyl saponins at the cellular level, we have prepared three fluorescent derivatives of dioscin and polyphyllin D (**A**−**C**). In this work, the fluorescent dansyl group was chosen because its emission properties are sensitive to the microenvironment.[7]

Scheme 1



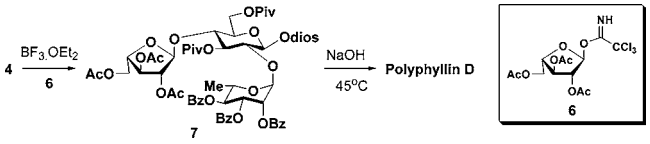
A modified synthetic route to dioscin and polyphyllin D has been developed in this work. As shown in Scheme 2, the synthesis was started from trillin (diosgenyl β-D-glucopyranoside) **1**.[6a,b,8] Treatment of **1** with pivaloyl chloride (PivCl) in CH_2Cl_2/pyridine (3:1) solution afforded the 3,6-di-Piv-protected product **2** in 85% yield. We found a higher product yield and regioselectivity than that reported in

Scheme 2



previous work[6b] could be obtained by carrying out the reaction at a lower temperature (−3 °C) and pyridine concentration (50%). TMSOTf-promoted glycosylation of diol **2** with 2,3,4-tri-*O*-benzoyl-α-L-rhamnopyranosyl trichloroacetimidate (**3**)[9] (1.2 equiv) gave the 2-*O*-glycosylated and 2,4-di-*O*-glycosylated products **4** and **5** in 36 and 42% yields, respectively. Subsequent base-mediated deprotection of **5** gave dioscin in 90% yield. As shown in Scheme 3,

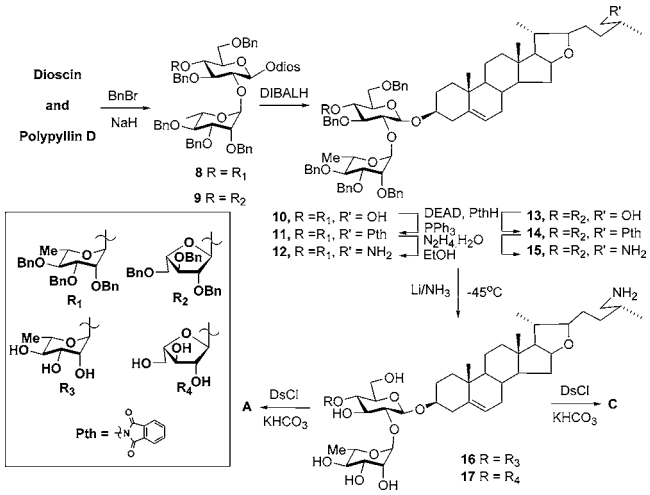
Scheme 3



Lewis-acid (BF_3·OEt_2)-catalyzed coupling of **4** with 2,3,5-tri-*O*-acytyl-α-L-arabinofuranosyl trichloroacetimidate **6** gave **7** in 87% yield.[6a,b] Deprotection of **7** under basic conditions with NaOH (12 equiv) afforded polyphyllin D in 91% yield.

The OH groups of dioscin and polyphyllin D were benzylated with BnBr and NaH to give **8** and **9** in 90 and 91% yields, respectively (Scheme 4). Treatment of **8** with

Scheme 4



DIBALH[10] afforded the dihydro (22*R*)-derivative **10** in 59% yield. Similarly, **13** was obtained in 61% yield from the reaction of **9** with DIBALH. The 26-hydroxyl primary alcohols **10** and **13** were readily converted to their phthal-

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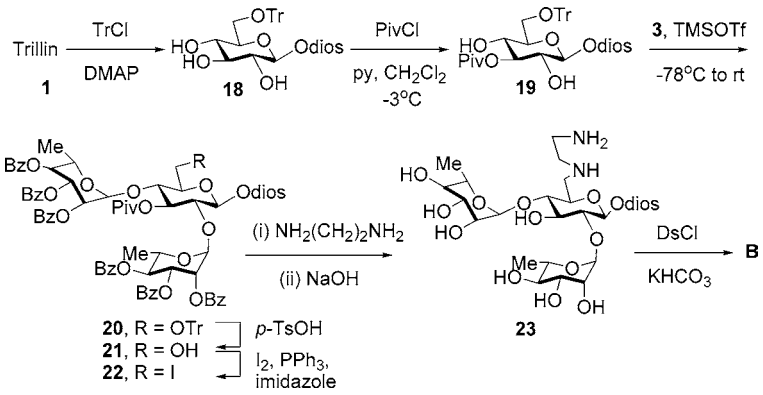
Scheme 5



imide derivatives by the Mitsunobu−Gabriel reaction[11] to give **11** (92% yield) and **14** (90% yield), respectively. Subsequent removal of the *N*-protecting group[12] with hydrazine hydrate and reduction of the benzyl groups[13] with lithium in liquid ammonia furnished the respective precursors **16** and **17** in 77 and 78% yields, respectively, over two steps. Reaction of **16** with dansyl chloride[14] (DsCl) and $KHCO_3$ afforded **A** in 77% yield. Under similar conditions, reaction of **17** with DsCl gave dansyl derivative **C** in 69% yield.

The synthesis of **B** is depicted in Scheme 5. First, **18** was prepared in 66% yield by treating trillin with triphenylmethyl chloride (TrCl) and (dimethylamino)pyridine in pyridine at 85 °C.[15] Reaction of **18** with PivCl in CH_2Cl_2/pyridine (3:1) solution afforded the 3-Piv product **19** in 82% yield.[16]

Glycosylation of diol **19** with **3** (2.5 equiv) catalyzed by TMSOTf gave the 2,4-di-*O*-glycosylated product **20** in 95% yield. The triphenylmethyl (Tr) protecting group was removed to give **21** in 80% yield.[17] Under the reaction conditions of I_2/PPh_3/imidazole,[18] the primary alcohol was converted to the iodide **22** in 86% yield. The ethylenediamine derivative **23** was prepared in 53% yield by refluxing **22** with 1,2-diaminoethane in THF and removing the Piv protecting group with NaOH. Condensation of **23** with DsCl and $KHCO_3$ gave **B** in 66% yield.

Dansyl derivatives **A**−**C** are strongly emissive. The absorption and emission spectra of **A** and **C** are essentially the same in DMSO solution, featuring an emission with λ_{max} at 514 nm [quantum yield = 0.4,[19] λ(excitation) = 339 nm] and two absorption bands at 262 (ϵ = 11 590 dm^3 mol^{-1} cm^{-1}) and 339 nm (ϵ = 3900 dm^3 mol^{-1} cm^{-1}). The dansyl derivative **B** emits at 518 nm [quantum yield = 0.39, λ (excitation) = 339 nm] and shows absorption maxima at 261 (ϵ = 12 800 dm^3 mol^{-1} cm^{-1}) and 339 nm (ϵ = 4600 dm^3 mol^{-1} cm^{-1}).

It has been reported that the dansyl fluorescence maximum λ_{max} increases with increasing polarity of the medium.[7] In this work, we studied the solvent effect on the emission properties of **A** and **B** in H_2O/DMSO (for **C**, the solvent effect and the emission properties are the same as those for **A**) and obtained some interesting findings.

As depicted in Figure 2, an increase in solvent polarity by changing the H_2O/DMSO ratio from 0 to 1:1 results in a red shift of λ_{max} of the dansyl fluorescence of **A** and **B** ($\Delta\lambda_{max(A)}$ = 17 nm, $\Delta\lambda_{max(B)}$ = 15 nm).

An interesting phenomenon was observed when the H_2O content was further increased. As depicted in Figure 2, the

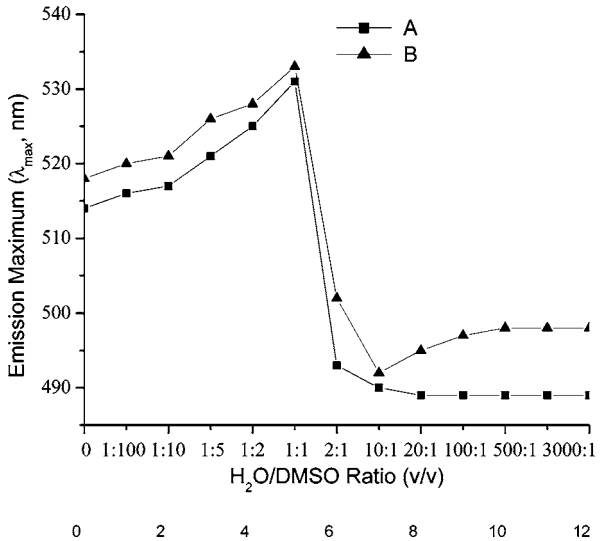


Figure 2. Change of the emission maximum of the dansyl derivatives **A** and **B** with changes in the solvent ratio of DMSO:H_2O.

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(17) Deprotection of **21** under basic conditions with NaOH (12 equiv) could afford dioscin. Thus, we could indirectly confirm the structure of **19**.

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fluorescence λ_{max} of **A** and **B** blue shifts abruptly once the H_2O:DMSO ratio is larger than 1:1. The λ_{max} of **A** was found to shift from 531 to 492 nm as the ratio of H_2O:DMSO increased from 1:1 to 2:1. A further increase in the H_2O:DMSO ratio did not have a significant effect as the ratio was gradually increased to more than 20:1, and the λ_{max} of **A** eventually stabilized at 489 nm. For **B**, its λ_{max} was found to blue shift from 533 to 492 nm as the ratio of H_2O:DMSO increased from 1:1 to 10:1. However, the change in the λ_{max} of **B** was dissimilar to that observed for **A** when the H_2O:DMSO ratio was altered from 10:1 to 3000:1. An increase of the H_2O:DMSO ratio from 2:1 to 500:1 was found to slightly increase the fluorescence λ_{max} of **B** from 492 to 498 nm. A further increase in the H_2O:DMSO ratio from 500:1 to 3000:1 did not have a significant effect, and the λ_{max} of **B** remained at 498 nm.

This phenomenon is unusual, as it is different from that reported in the literature.[7] A decrease in λ_{max} suggests that the dansyl group is located in a less polar environment. On this premise, we propose that **A** and **B** form dispersed micelles when the H_2O:DMSO ratio is larger than 1:1; the outer part of the micelle consists of hydrophilic sugar moieties, and the hydrophobic aglycone and dansyl groups form the inner core of the micelle. Thus, the dansyl groups become surrounded by a nonpolar environment. Two possible micellar models are depicted in Figure 3.

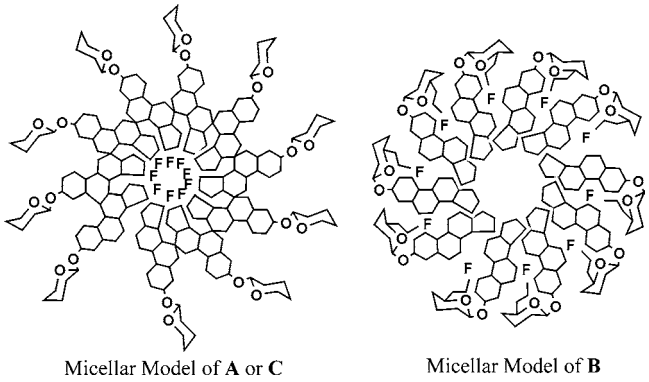

Figure 3. Possible micellar models of **A**−**C**. Abbreviation: F = fluorophore moiety.

In the case of **A**, as the ratio of H_2O:DMSO increases above 20:1, micellar self-assembly occurs in a manner where the dansyl groups are positioned at the center of the micelle, presumably to minimize further changes in solvent polarity. As depicted in Figure 3, the dansyl groups of **B** are proposed to be located on the outer rim of the micellar structure. This arrangement would permit a response to changes in the solvent environment even after the micelles completely formed.

The cytotoxicity of dioscin, polyphyllin D, and the dansyl derivatives **A**−**C** against the HeLa cell line has been evaluated by standard MTT assay,[20] and the results are depicted in Table 1.

Table 1. Cytotoxicities (IC_{50}) of Dioscin, Polyphyllin D, and Their Dansyl Derivatives against HeLa Cells[a]

compound	IC_{50} (μM) in 72 h
dioscin	3.8 ± 0.3
polyphyllin D	1.1 ± 0.02
A	15.7 ± 0.6
B	15.3 ± 0.2
C	17.6 ± 0.5
cisplatin	7.4 ± 0.1

[a] HeLa: cervix epitheloid carcinoma.

The cytotoxic activities of dansyl derivatives **A** (IC_{50} = 15.7 ± 0.6 μM) and **C** (IC_{50} =17.6 ± 0.5 μM) are lower than those of their parent compounds (dioscin, IC_{50} = 3.8 ± 0.3 μM; polyphyllin D, IC_{50} = 1.1 ± 0.02 μM) on the basis of IC_{50} values. This suggests that the F ring of the steroid and spirostanol structural unit plays an important role in the cytotoxicities toward HeLa cells. When the spirostanol structural unit was lost, the two saponins lost considerable efficiency. Similar findings had been reported for steroidal saponins.[4b,d]

The cytotoxic activity of dansyl derivative **B** (IC_{50} = 15.3 ± 0.2 μM) is also less toxic than its parent compound, dioscin. We conceive that attachment of a hydrophobic dansyl group at the sugar terminal of dioscin might lower its hydrophilic character, which results in a higher IC_{50} value.

In conclusion, we have synthesized three fluorophore-appended steroidal saponin derivatives of dioscin and polyphyllin D and examined their micellar properties and bioactivities. Efforts are currently under way to examine the intercellular target and actions of the two saponins by in vitro and in vivo studies.

Acknowledgment. This work is supported by the Area of Excellence Scheme (AoE/P-10-01) established under the University Grants Committee, HKSAR, the Hong Kong Research Grants Council (HKU7039/03P), HKSAR, and The University of Hong Kong (University Development Fund).

Supporting Information Available: Experimental details for the synthesis of **A**−**C** as well as their cytotoxicity tests and analytical data for new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

OL0475616

(19) Fluorescence quantum yields (F_f) of **A**−**C** were determined by using quinine sulfate in 0.1 N H_2SO_4 as the reference standard (F_f = 0.546).

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