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Rapid Communication

# Synthesis of monomethylated dioscin derivatives and their antitumor activities

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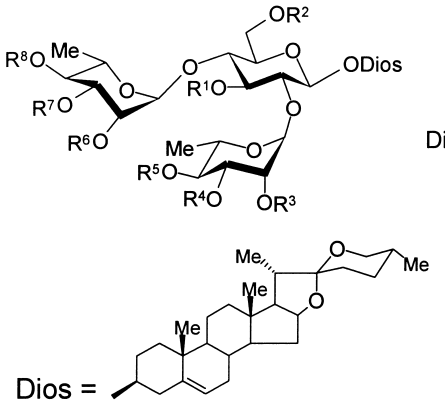
**Abstract**

All possible eight monomethylated dioscin derivatives (**1**–**8**) were synthesized. Their inhibitory activities against P388 and A-549 cells were determined, and the results indicate that six of the eight hydroxyls of dioscin are the 'key polar groupings' for tumor inhibitory activities. 

*Keywords:* Dioscin; Methylation; Synthesis; Antitumor

Spirostan saponins are the largest group of steroidal saponins occurring in plants.[1] While the spirostan aglycones provide a rigid bulky moiety, the glycoforms render a relatively mobile conformation for the molecule. A quite common feature of spirostan saponins is their inhibitory activities against tumor cells, with their $IC_{50}$s being at the μM level. Dioscin, diosgenin-3-yl α-L-rhamnopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→4)]-β-D-glucopyranoside, represents a typical example of spirostan saponins. In fact, dioscin is one of the most common saponins occurring in plants that has been isolated from some twenty genera. Many of these plants are vegetables or traditional medicinal plants, especially from the Orient. Besides antitumor activities,[2,3] antiviral,[4] antifungal,[5] antiinflammatory,[6–8] and immunostimulant activities[9] were also observed for this spirostan saponin. Recently, synthetic approaches toward dioscin and its congeners have been developed[1b,10] that give access to their derivatives for developing more potent compounds, as well as probing structure–activity relationships and deciphering biological mechanisms.




| | R1 | R2 | R3 | R4 | R5 | R6 | R7 | R8 |
|---|---|---|---|---|---|---|---|---|
| Dioscin | H | H | H | H | H | H | H | H |
| **1** | Me | H | H | H | H | H | H | H |
| **2** | H | Me | H | H | H | H | H | H |
| **3** | H | H | Me | H | H | H | H | H |
| **4** | H | H | H | Me | H | H | H | H |
| **5** | H | H | H | H | Me | H | H | H |
| **6** | H | H | H | H | H | Me | H | H |
| **7** | H | H | H | H | H | H | Me | H |
| **8** | H | H | H | H | H | H | H | Me |

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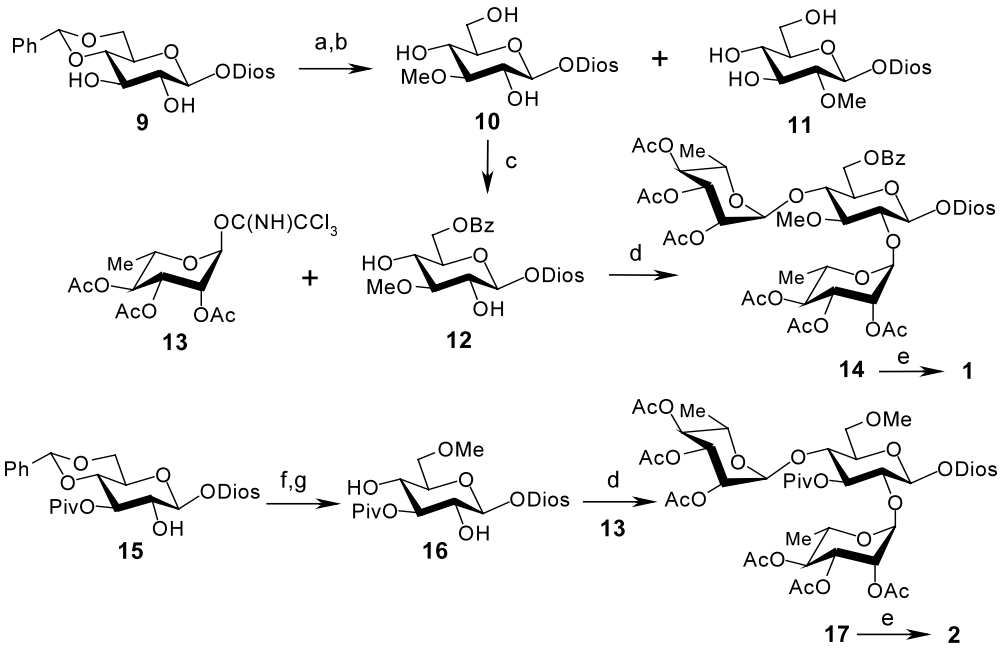
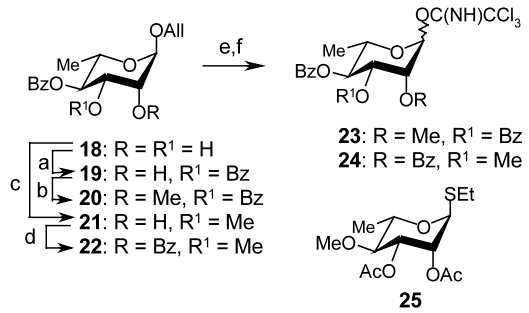


Scheme 1. Reagents and conditions: (a) $Bu_2SnO$, PhMe, then $CH_3I$, $t$-$Bu_4N^+Br^-$, 78%; (b) $p$-TsOH, MeOH–$CH_2Cl_2$, 40% for **10**; 46% for **11**. (c) BzCl, pyridine, −40 °C, 81%; (d) **13** (4.0 equiv), TMSOTf (0.2–0.3 equiv), $CH_2Cl_2$, 81% for **14**; 92% for **17**. (e) MeONa, MeOH–$CH_2Cl_2$, 76% for **1**; 78% for **2**. (f) $p$-TsOH, MeOH–$CH_2Cl_2$, 76%. (g) $(Bu_3Sn)_2O$, PhMe; then $CH_3I$, $t$-$Bu_4N^+Br^-$, 29%.

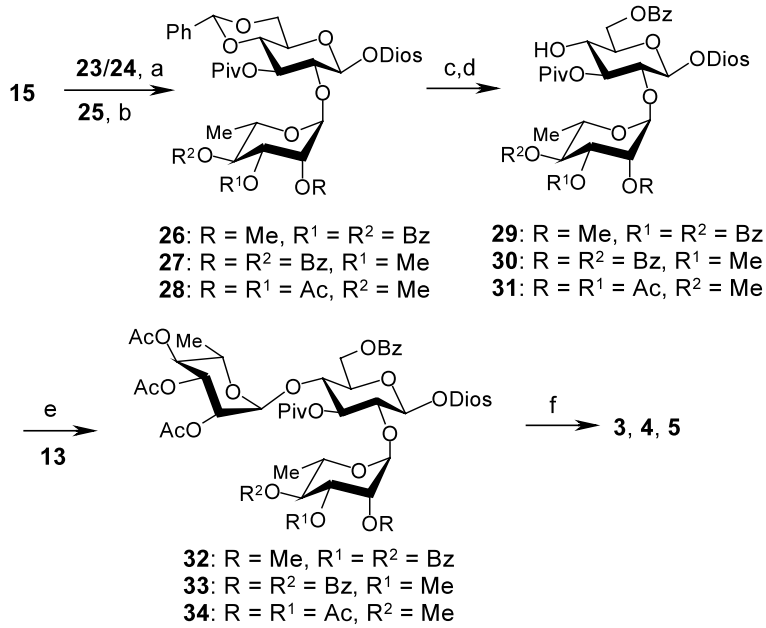
To find a starting point for derivatization, we managed to prepare all possible monomethylated dioscins (**1**–**8**). Replacement of a hydroxyl group on a sugar with a methoxy group has been an effective strategy to probe the involvement of a particular hydroxyl group in the recognition of the parent sugar on target proteins.[11,12] For example, substitution of a 'key polar hydroxyl group'[13] with a methoxy group on a disaccharide substrate of a glycosyltransferase completely blocked their recognition;[11] while substitution of all the hydroxyl groups with methoxy groups on the heparin pentasaccharide did not affect its binding with antithrombin III, demonstrating no 'key polar hydroxyl groups' are involved in the interaction of heparin with antithrombin III.[12]

Along the versatile synthetic route toward dioscin and its congeners,[10] the monomethylated derivatives (**1**–**8**) were readily synthesized as shown in Schemes 1–4. Compounds **1** and **2** have the methoxy groups substituted on the core glucose residue and were prepared starting from diosgenyl glucopyranosides **9**[10] and **15**[10] (Scheme 1). Compounds **3**–**8** have the methoxy groups substituted on the peripheral rhamnose residues. Their preparation thus requires the glycosylation of the corresponding diosgenyl glucopyranoside monols (**15** and **35**[10]) with monomethylated rhamnopyranosyl donors (Schemes 3 and 4). Rhamnosyl donors **23** and **24** were readily prepared as shown in Scheme 2, and donor **25** was readily prepared from ethyl 4-*O*-methyl-1-thio-α-L-rhamnopyranoside[14] by acetylation.

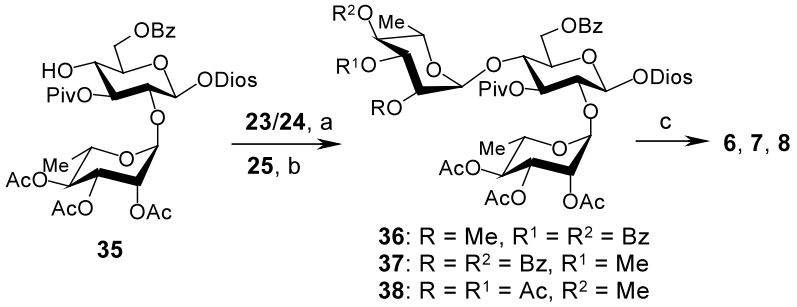
Since the present syntheses adopted modifications of the well-documented synthetic route toward dioscin and its congeners,[10] the chemistry worth discussion here is the organotin-mediated regioselective methylation[15] and glycosylation with monomethylated rhamnopyranosyl donors. Regioselective benzoylation or methylation of the $2_{ax}$,$3_{eq}$-diol **18** via a dibutylstannylene intermediate gave the $3_{eq}$-substituted products (**19** and **21**) in excellent yields. Methylation of $2_{eq}$,$3_{eq}$-diol **9** under similar conditions gave no regioselectivity, leading to, after cleavage of the 4,6-*O*-benzylidene group, 3-OMe and 2-OMe products **10** and **11** in 1:1.2 ratio. Attempts at selective methylation of the primary 6-OH of a triol to obtain **16** via tributylstannyl ether intermediates gave



Scheme 2. Reagents and conditions: (a) $Bu_2SnO$, PhMe, 140 °C; then BzCl, pyridine, rt, 71%. (b) $CH_2N_2$, $BF_3{\cdot}OEt_2$, $CH_2Cl_2$, rt, 87%. (c) $Bu_2SnO$, PhMe, 140 °C; then $CH_3I$, $t$-$Bu_4N^+Br^-$, rt; (d) BzCl, pyridine, rt, 80% (for 2 steps). (e) $PdCl_2$, MeOH, rt; (f) $CCl_3CN$, DBU, $CH_2Cl_2$, rt, 76% for **23**; 59% for **24** (2 steps).




Scheme 3. Reagents and conditions: (a) **23**/**24** (3.0 equiv), TMSOTf (0.05 equiv), $CH_2Cl_2$, 80% for **26**; 72% for **27**. (b) **25** (3.0 equiv), NIS/AgOTf, $CH_2Cl_2$, 81% (for **28**). (c) *p*-TsOH, MeOH–$CH_2Cl_2$, 40 °C; (d) BzCl, pyridine, 0 °C, 78% for **29**; 76% for **30**; 66% for **31** (2 steps). (e) **13** (3.0 equiv), TMSOTf (0.05 equiv), $CH_2Cl_2$, rt, 66% for **32**; 76% for **33**; 79% for **34**. (f) NaOMe, MeOH–$CH_2Cl_2$, 72% for **3**; 80% for **4**; 89% for **5**.




Scheme 4. Reagents and conditions: (a) **23**/**24** (3.0 equiv), TMSOTf (0.05 equiv), $CH_2Cl_2$, 85% for **36**; 96% for **37**. (b) **25** (3.0 equiv), NIS/AgOTf, $CH_2Cl_2$, 84% (for **38**). (c) NaOMe, MeOH–$CH_2Cl_2$, 73% for **6**; 80% for **7**; 75% for **8**.

Table 1
Growth inhibition rate (%) of monomethylated dioscin derivatives **1**–**8** on tumor cells (P388 and A-549)

| Compound | P388 | | | A-549 | | |
|---|---|---|---|---|---|---|
| | $10^{-5}$ M | $10^{-6}$ M | $10^{-7}$ M | $10^{-5}$ M | $10^{-6}$ M | $10^{-7}$ M |
| **1** | 16.8 | 0 | 0 | 36.5 | 1.9 | 0 |
| **2** | 100 | 90.6 | 4.4 | 99.5 | 69.4 | 0 |
| **3** | 100 | 16.1 | 0 | 99.3 | 15.7 | 0 |
| **4** | 54.6 | 0 | 0 | 99.3 | 34.3 | 8.9 |
| **5** | 94.8 | 0.9 | 0 | 97.4 | 7.4 | 7.9 |
| **6** | 91.4 | 12.1 | 2.2 | 72.3 | 0 | 0 |
| **7** | 97.7 | 22.3 | 5.6 | 99.6 | 10.4 | 0 |
| **8** | 99.4 | 74.9 | 0 | 99.6 | 63.5 | 0 |
| Adriamycin | 100 | 100 | 100 | | 70.2 | 55.9 |

the desired **16** as a major product, albeit in only 29% yield. In addition, methylation of the monol **19** was not successful under many of the usual conditions, e.g., MeI/$Ag_2O$/DMF, MeOTf/DTBMP, due to the neighboring acyl group migrations. Methylation was finally effected by means of $CH_2N_2$ in the presence of $BF_3{\cdot}OEt_2$, giving **20** in 87% yield. Glycosylation with monomethylated rhamnopyranosyl trichloroacetimidates **23** and **24** and thioglycoside **25** under the promotion of TMSOTf and NIS/AgOTf, respectively, afforded the expected products (**26**–**28** and **36**–**38**) in satisfactory yields (66–96%). Especially, glycosylation with donor **23**, in the absence of neighboring group participation at the C-2 position, provided only the α products (**26** and **36**), reflecting a strong anomeric effect in the glycosylation with L-rhamnopyranosyl donors.[16] In comparison, the monomethylated rhamnopyranosyl donors **23**–**25** demonstrated more reactivity than the peracetylated trichloroacetimidates **13**, producing the corresponding glycosylation products, in glycosylation with the 4-OH of the disaccharides **29**–**31** and **35**, in higher yields (cf., 66–79% yield for **32**–**34**; 84–96% yield for **36**–**38**).

The in vitro inhibitory activities of the monomethylated dioscins **1**–**8**[†] against the growth of P388 (mouse leukemia) and A-549 (human lung adenocarcinoma) were evaluated by the standard MTT assay.[17] The results were listed in Table 1. In comparison with dioscin, which has an $IC_{50}$ of 0.46 μM against P388,[2] the activities of the monomethylated compounds **2** and **8** were largely retained, while the inhibitory activities of the other six compounds were considerably decreased. These results indicated that the six hydroxyl groups (OH-1 and OH-3–OH-7) on dioscin might be the 'key polar hydroxyl groupings'[13] contributing to its antitumor activity. Thus the OH-2 and OH-8 could be the sites for labeling to provide derivatives for further mechanistic studies. In fact, the OH-2 and OH-8 of dioscin and its congeners have been demonstrated to be the most distant hydroxyl groups from the aglycone. Thus these could be regioselectively acylated by Novozyme 435. The resulting monoacetylated derivatives showed similar antitumor activities as the parent compounds.[18] Those findings are in agreement with the present results.

## Acknowledgements

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[†] The structures of **1**–**8** were unambiguously determined by extensive 2D NMR analysis and were further confirmed by ESIMS and elemental analysis.

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