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Synthesis of a Potent and Selective Inhibitor of p90 Rsk

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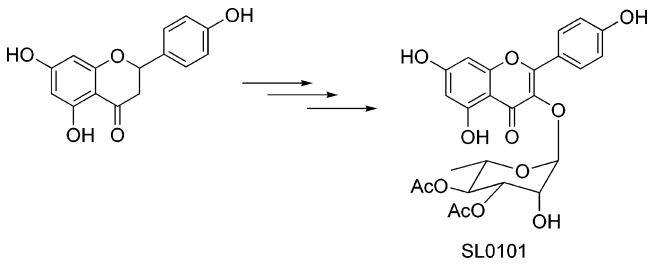
ABSTRACT



SL0101

The synthesis of the naturally occurring kaempferol glycoside SL0101 has been accomplished, as has its biochemical evaluation. SL0101 exhibits selective and potent p90 Rsk inhibitory activity at nanomolar concentrations without inhibiting the function of upstream kinases such as MEK, Raf, or PKC. The synthesis verified the structural assignment of the natural product and has provided access to material sufficient for detailed biological evaluation.

The overexpression of proteins in the mitogen-activated protein kinase (MAPK) pathway has been noted for a number of human malignancies,[1] suggesting the possible utility of inhibitors of these proteins in cancer therapy. However, MAPK is involved in many fundamental cellular processes such as apoptosis, survival, differentiation, and proliferation.[2] Consequently, the inhibition of protein mediators involved in multiple processes may result in effects on both normal and cancer cells in a nonselective manner. Logically, the identification of downstream mediators in the MAPK pathway essential for tumor growth might lead to inhibitors capable of selectively targeting cancer cells.

Rsks are a family of 90 kDa ribosomal S6 kinases, which are downstream effectors of MAPK; to date, four isoforms have been identified.[3] Recent studies have demonstrated the role of Rsk in cell survival signaling via the phosphorylation and inactivation of the proapoptotic protein BAD. Rsk has also been shown to directly promote cell survival by regulating the expression and activation of prosurvival proteins such as CREB (cyclic adenosine monophosphate response element binding protein).[4] The combination of promoting cell survival and prevention of apoptosis causes excessive cell survival, eventually leading to diseases such as cancer and autoimmune disorders.[4b] Additionally, it has been found that Rsk2 is overexpressed in more than 50% of human breast cancers, validating the Rsk family as a potential target for drug design.

Recently, we described three kaempferol glycosides, isolated from a methanol extract of *Forsteronia refracta*, that selectively inhibit p90 Rsk (Figure 1).[5] Interestingly, SL-

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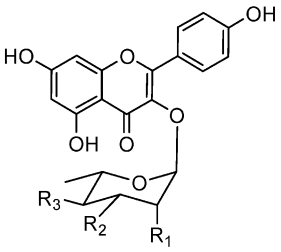


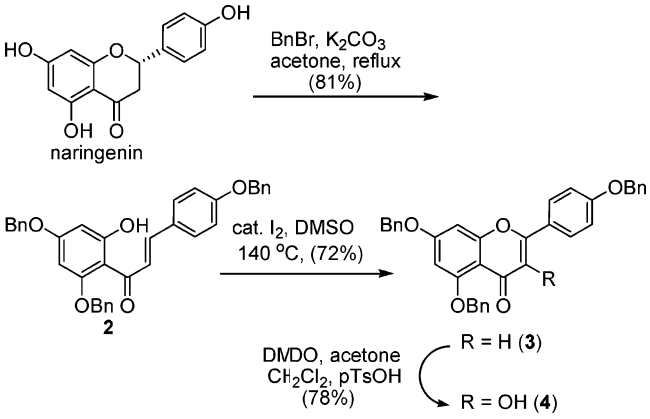
	R_1	R_2	R_3	IC_{50}
SL0101 (**1**)	OH	OAc	OAc	89 nM
4"-*O*-acetyl SL0101	OH	OH	OAc	189 nM
2",4"-di-*O*-acetyl SL0101	OAc	OH	OAc	580 nM

Figure 1. Structures of SL0101 (**1**), 4″-*O*-acetyl SL0101, and 2′,4″-*O*-diacetyl SL0101 and their Rsk2 inhibitory activities.

0101 (**1**) inhibited Rsk1 and Rsk2 to a greater extent than Rsk3, although Rsk2 and Rsk3 are 80% homologous at the level of primary sequence. Further, in comparison to its potent inhibition of Rsk2 (IC_{50} ~89 nM), **1** was found not to inhibit upstream kinases such as MEK, Raf, and PKC.[5] The interesting biological activities of **1**,[5] and its limited availability from natural sources, prompted an investigation into the synthesis of **1**.

The short and convergent synthetic approach to **1** started with the preparation of flavonol **4**, as outlined in Scheme 1.

Scheme 1



Naringenin (4′,5,7-trihydroxyflavanone) was treated with benzyl bromide and excess K_2CO_3, resulting in concomitant β-elimination and benzyl protection to give the chalcone **2** in 81% yield. Formation of the desired flavone **3** was accomplished in good yields (70−80%) using catalytic I_2 in DMSO at 140 °C.[6] Introduction of a 3-OH group was achieved using dimethyldioxirane (DMDO),[7] followed by opening of the formed epoxide with catalytic *p*-toluenesulfonic acid to afford flavonol **4** in 78% yield.[8]

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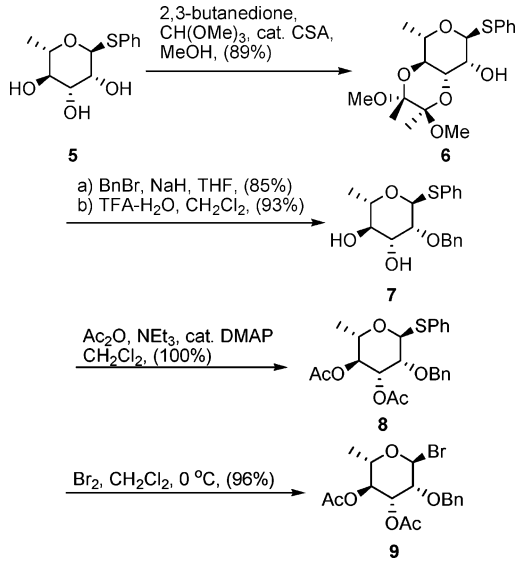
Preparation of the carbohydrate moiety is outlined in Scheme 2. Compound **5** was synthesized from L-rhamnose

Scheme 2



by known methods.[9] Since O-3 and O-4 of **1** are both acetylated, while O-2 is unprotected, we sought an appropriate orthogonal protecting group for O-2. Accordingly, using a procedure reported by Crich and co-workers,[10] regioselective protection of the O-3 and O-4 hydroxyl groups was achieved using 2,3-butanedione and trimethylorthoformate to give **6** in 89% yield. Benzyl protection of O-2 proceeded in 85% yield via the agency of NaH and benzyl bromide in THF to give the fully protected rhamnose. Benzylation of this OH group was chosen to allow for a mild and efficient global deprotection at the end of the synthesis. Removal of the O-3,4 protecting group was accomplished using TFA−H_2O in CH_2Cl_2 to afford **7** in 93% yield. Bis-acetylation of **7** with Ac_2O, NEt_3, and catalytic 4-(dimethylamino)pyridine (DMAP) gave 2-*O*-benzyl-3,4-di-*O*-acetylrhamnose derivative **8** in yields exceeding 90%. Conversion to the rhamnosyl bromide **9** was accomplished in 84% yield by treatment with Br_2 in CH_2Cl_2 at 0 °C. Condensation of **4** and **9** in the presence of Ag_2O provided perbenzylated SL0101 (**10**), exclusively as the α-anomer, in 60% yield (Scheme 3). Other commonly used glycosylation methods[11] such as benzyltriethylamine bromide and dilute aqueous KOH failed. Global debenzylation using Pearlman's catalyst ($Pd(OH)_2$/C) in the

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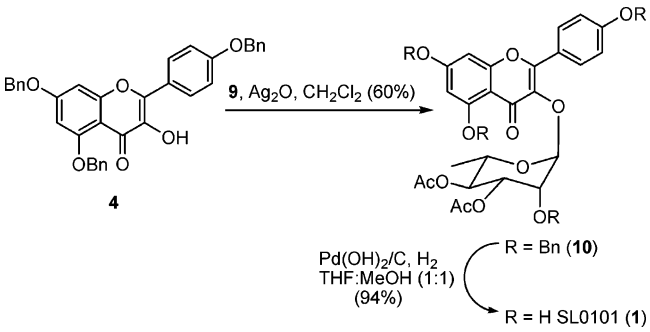
Scheme 3



presence of H_2 gave SL0101 (**1**) in 94% yield. The synthetic compound had spectral data in full agreement with the authentic, naturally derived sample.

In conclusion, a short and efficient approach to the naturally occurring p90 Rsk inhibitor **1** has been completed. This convergent approach has permitted access to **1** on a preparative scale and should provide facile access to structurally related analogues. Further investigations into the biological activity of **1** are currently underway.

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Supporting Information Available: Experimental procedures and characterization data for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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