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Stereoselective Synthesis and Evaluation of C6″-Substituted 5a-Carbasugar Analogues of SL0101 as Inhibitors of RSK1/2

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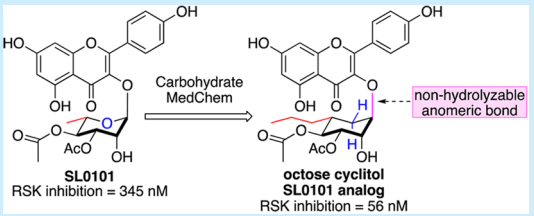
Supporting Information

ABSTRACT: A convergent synthesis of 5a-carbasugar analogues of the *n*-Pr-variant of SL0101 is described. The analogues were synthesized in an effort to find compounds with potent *in vivo* efficacy in the inhibition of p90 ribosomal s6 kinase (RSK1/2). The synthesis derived the desired *C*-4 L-rhamnose stereochemistry from quinic acid and used a highly selective cuprate addition, $NaBH_4$ reduction, Mitsunobu inversion, and alkene dihydroxylation to install the remaining stereochemistry. A Pd-catalyzed cyclitolization stereoselectively installed the aglycon at the anomeric position. The analogues were evaluated as RSK1/2 inhibitors and found to have 3- to 6-fold improved activity.

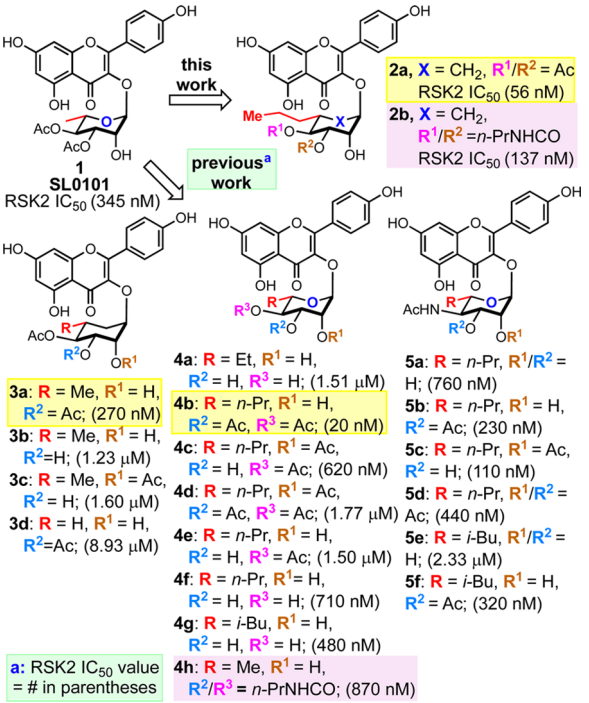


The p90 ribosomal s6 kinases (RSK) are a family of Ser/Thr protein kinases.[1] Two isoforms (RSK1/2) from this family are involved in the etiology of a number of different cancers.[2] In an effort aimed at identifying RSK1/2 inhibitors, the flavonoid glycoside natural product SL0101 (**1**) was discovered as a relatively selective inhibitor of the *N*-terminal kinase domain (NTKD) of RSK.[3] RSK has two kinase domains where the N-terminal domain (NTKD) is responsible for phosphorylation of target substrates.[4] Based on the crystal structure of the RSK2 NTKD complexed with SL0101, a major conformational rearrangement of the N-lobe of the kinase domain generates the inhibitor-binding pocket.[5]

Inspired by its unique activity and selectivity, we have been exploring structure−activity relationship (SAR) requirements associated with SL0101 (**1**).[3,6] As part of these studies, we have developed a *de novo* asymmetric synthesis[7] of SL0101, its enantiomer, and several congeners.[8] Our studies have identified several analogues with improved activity and have emphasized the importance of the rhamnose sugar and its *C*-3 and *C*-4 acetates. In addition, we have found that substitution at the *C*-6 position[8,9] and the ring oxygen (**2** and **3**) of the sugar gives improved efficacy in the *in vitro* kinase assays and cell-based studies.[8,10]

SL0101 (**1**) has a short biological half-life *in vivo*,[5] which is presumably due to the hydrolyzable *C*-3/*C*-4-acetates on the sugar, as well as an *O*-glycosidic bond. To identify less labile groups that could replace the ester without loss of affinity, we have investigated replacing the rhamnose *C*-4-acetate (e.g., **5a−f** with a *C*-4 acetamide), the *C*-3/*C*-4-acetates (e.g., **4h** with a *C*-3/*C*-4-*n*-Pr-carbamates), and the ring oxygen with a methylene group (i.e., **3a−d** carbasugars).[8] As part of our ongoing effort to identify RSK1/2-inhibitors as potential therapeutics, we decided to test the effects of combining these three substitutions and targeted two analogues **2a−b** (Scheme 1). Herein we disclose the synthesis of cyclitol analogues **2a** and **2b** as well as the relative RSK2 inhibitory activity.[11]

Scheme 1. SAR for SL0101 RSK Inhibition[8]



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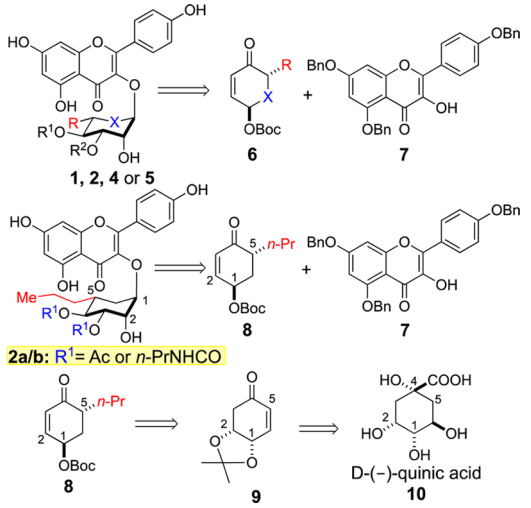
Retrosynthetically, we envisioned preparing **2a** and **2b** in a route analogous to our previously established routes to SL0101 analogues **1**–**5** (e.g., **6** + **7**, Scheme 2). Specifically, we expected

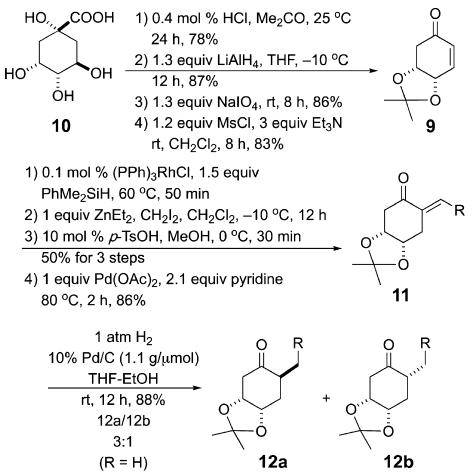
Scheme 2. Retrosynthesis of *n*-Pr Carbasugar Analogues



that **2a**/**b** would arise from the Pd-catalyzed cyclitolization of **7** with enone **8**.[8,12] Base on our previous success, we viewed the cyclitol donor **8** would arise from quinic acid **10** via enone **9**,[13] where the quinic acid tertiary alcohol would become the *C*-4 ketone and the central alcohol of the triol would become the *C*-1 anomeric position.

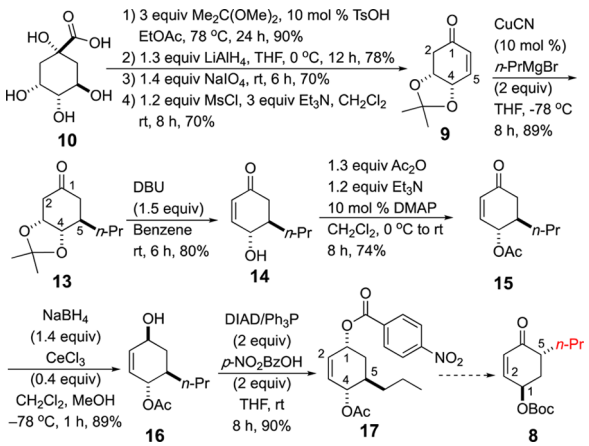
At the outset, our efforts to extend our previous cyclitol synthesis (R = H) encountered difficulty, associated primarily with achieving high stereocontrol in the conversion of **9** into **12b**. Specifically, we explored the possibility of installing the sugar L-stereochemistry at *C*-5 by means of a selective hydrogenation of the enone **11** from the exoface to selectively provide **12b** over **12a**. To our surprise, when we exposed **11** to typical hydrogenation conditions (1 atm of H_2 with Pd/C), we found **12a** was the major isomer, but with poor diastereoselectivity (3:1) (Scheme 3). This problem was exacerbated by the fact that isomerization of the position α to the ketone occurred when we tried to deprotect the acetonide.[12] These two compounding factors prompted a search for an alternative approach.[14]

Scheme 3. Initial Attempt to α-L-Cyclitol Donors



Our redesigned retrosynthesis turned the original design 180° (Scheme 4), with the quinic acid tertiary alcohol becoming the

Scheme 4. Synthesis of Pd-Cyclitolization Donor



carbasugar *C*-1 anomeric position and the central alcohol of the triol becoming the *C*-4 enone **8**. Because of complications with its conversion to enone **8** this effort turned to the synthesis of allylic acetate **17**. Specifically, the allylic benzoate **17** already had a Pd-π-allyl leaving group at the pseudoanomeric position for the cyclitolization reaction. In addition, benzoate **17** also had the desired carbasugar *C*-4 acetate in the correct *rhamno*-stereochemistry. The question that remained was could we find conditions to selectively ionize the axial *C*-1 allylic *p*-NO_2Bz group without touching the allylic *C*-4 acetate. In addition, we were concerned that the *C*-4 acetate may not control the regiochemistry of nucleophilic attack to the π-allyl intermediate (*i.e.*, **TS-1**) as well as the *C*-4 ketone (*i.e.*, **TS-2**).[15] In general, we found a *C*-4 ketone both improved the electrophilicity of the Pd-π-allyl intermediate and helped direct nucleophilic addition to the *C*-1 position. In this regard, we were hopeful that the regiocontrol issues could also be controlled by the *C*-5 *n*-propyl substituent.[16] Altenatively, chiral ligands on the Pd-π-allyl could be used if the *C*-4 acetate is not sufficient for controlling the regiochemistry.

Our redesigned synthesis returned to enone **9**, which underwent a highly stereoselective cuprate-promoted addition of *n*-propyl anion to furnish ketone **13** in an excellent yield and as a single diastereomer (89%).[17] Exposure of **13** to DBU in benzene gave allylic alcohol **14** in good yield (80%). The required *C*-4 acetate was then installed by an acylation of **14** with acetic anhydride/DMAP with Et_3N to deliver allylic acetate **15** (74%). A stereoselective 1,2-reduction of enone **15** under Luche conditions[18] gave the allylic alcohol **16** in good yield (89%) and diastereoselectivity (>10:1). Analysis of the allylic coupling constants in the ^{1}H NMR for **16** indicated that the Luche reduction occurred via axial attack to install an equatorial alcohol,[19] which corresponds to β-anomeric stereochemistry in the resulting carbasugar. Thus, a Mitsunobu reaction was preformed on **16** with DIAD/PPh_3 and *p*-nitrobenzoic acid to yield the desired cyclitol donor **17** in excellent yield (90%).[20]

Unfortunately, the conversion of the bis-allylic ester **17** to enone **8** proved to be untenable, as conditions for the selective hydrolysis of either of the two esters were not found. In an effort to find a viable alternative, we decided to explore the use of allylic *p*-NO_2-benzoate **17** as a cyclitol donor, in the cyclitolization reactions. The allylic *p*-nitrobenzoate **17** had some potential

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advantages to **8** in our planned synthesis of SL0101 analogue **2a**. Specifically, the *C*-1 allylic-benzoate **17** already had a Pd-π-allyl leaving group at the anomeric position for the cyclitolization reaction. In addition, the *p*-nitrobenzoate **17** also had the desired *C*-4 acetate with the correct stereochemistry installed, and thus, the aglycon portion would go through two less transformations (Figure 1).

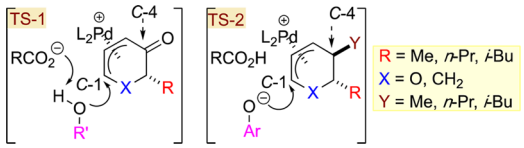
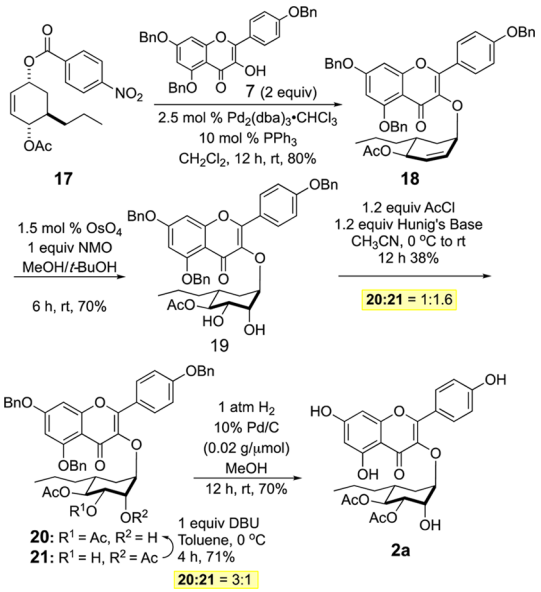


Figure 1. Competing Pd-π-allyl mechanisms.

To our delight, when a mixture of *p*-nitrobenzoate **17** and flavonol **7** was exposed to our typical glycosylation conditions (2.5 mol % $Pd_2(dba)_3$·$CHCl_3$ and 10 mol % of PPh_3 in CH_2Cl_2 at 0 °C) the reaction proceeded smoothly to provide the desired product in good yield (80%), in excellent regio- and stereoselectivity (Scheme 5). Exposure of the allylic acetate **18** to the

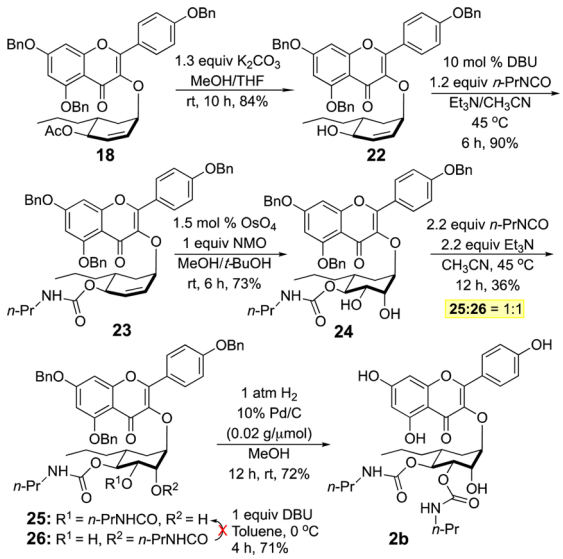
Scheme 5. Synthesis of *n*-Pr Carbasugar SL0101 Analogue



Upjohn conditions[21] (OsO_4/NMO; 70%) stereoselectively converted it into the *rhamno*-diol **19** which, when acylated with acetyl chloride and Hunig's base, gave a mixture of the *C*-3 and *C*-2 acetates **20** and **21** (1:1.6) in 38% yield. Fortunately, the undesired *C*-2 acetate **21** could be isomerized to a mixture of acetates which favored the desired *C*-3 acetate **20** (3:1). Finally, the desired regioisomer **20** was globally deprotected by an exhaustive hydrogenolysis (1 atm of hydrogen with Pd/C), which produced the target *C*-3/*C*-4 diacetate **2a** in good yield (70%).

With access to the desired diacetate **2a** having been established, we turned our attention to the second target compound biscarbamate **2b** (Scheme 6). Our approach to biscarbamate **2b** returned us to allylic acetate **18**, which could be hydrolyzed into allylic alcohol **22** with K_2CO_3 in methanol (84%). The allylic alcohol **22** was converted into *n*-Pr-carbamate **23** by exposure of it to *n*-Pr-isocyanate and base (10% DBU/

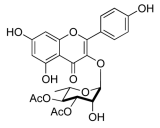
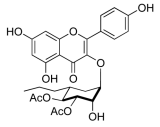
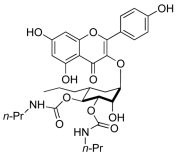
Scheme 6. Synthesis of Bis-carbamate Analogues



Et_3N in MeCN). Exposure of the allylic carbamate **23** to the Upjohn conditions (OsO_4/NMO; 73%) stereoselectively converted it into the *rhamno*-diol **24**. Re-exposure of the *C*-2/*C*-3 diol **24** to the carbamate forming conditions (*n*-Pr-isocyanate, 10% DBU/Et_3N in MeCN) gave a mixture of *C*-3 and *C*-2 carbamates **20** and **21** (1:1). The desired *C*-3/*C*-4 biscarbamate **25** could be isolated from that mixture in a 40% overall yield. Unfortunately, the undesired *C*-2/*C*-4 biscarbamate **26** could not be isomerized under basic conditions as was the case with the diacetate. Finally, the desired *C*-3/*C*-4 regioisomer **25** was globally deprotected by an exhaustive hydrogenolysis (1 atm of hydrogen with Pd/C), to afford the target *C*-3/*C*-4 biscarbamate **2b** in good overall yield (72%).

The efficacy of the two cyclitol analogues **2a** and **2b** to inhibit RSK2 activity was determined in *in vitro* kinase assays using purified recombinant RSK2 (Table 1).[3] The data were fit using nonlinear regression analysis. Both SL0101 analogues showed improved RSK inhibition over the lead structure SL0101, the *n*-Pr cyclitol diacetate **2a** having a >6-fold decrease in IC_{50} (54 nM), whereas the bis-carbamate **2b** had an ~3-fold decrease in

Table 1. *In Vitro* Potency of SL0101 (1) and Analogues[a]

Analogue		RSK2 IC_{50} [nM]
SL0101 (**1**)[b]		345 ± 100
2a		56 ± 27
2b		137 ± 59

[a]For procedure see Supporting Information. [b]See ref 11.

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IC_{50} (137 nM). In the parent series, cyclitol substitution (i.e., **1** to **3a**) led to a modest improvement in the inhibitory activity (345 to 270 nM). In the *n*-Pr-series, however, the effect of cyclitol substitution (i.e., **4b** to **2a**) trended toward reduced efficacy (20 to 56 nM). This loss in activity should be easily compensated for by the expected improved bioavailability that results from the hydrolysis-resistant cyclitol substitution. There was also a loss in inhibitory activity (345 to 870 nM) by the *n*-Pr-carbamate substitution in the parent series (**1** to **4h**). This negative effect was also observed with the cyclitol series (i.e., **2a** to **2b**: 56 to 137 nM).

In conclusion, using a highly stereoselective Pd-cyclitolization reaction, two new cyclitol analogues of the natural product SL0101 were synthesized and evaluated. These analogues **2a** and **2b** showed significant improvement in RSK inhibitory activity. Improved bioavailability has already been shown for **2a**.[11] The synthesis of these new SL0101 cyclitol analogues required the discovery of a novel synthesis of a new cyclitol donor, which demonstrated that the previously believed requirement of a *C*-4 ketone in the cyclitol donor is not necessary for the reaction with phenol-like nucleophiles. Further studies aimed at defining the requirements for a specific-RSK1/2 inhibition are ongoing and will be reported in due course.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.7b00945.

Experimental procedures and spectral data (PDF)

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Notes

The authors declare the following competing financial interest(s): The coresponding authors Deborah A. Lannigan and George A. O'Doherty have applied for patent protection for this class of compounds.

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