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SHORT COMMUNICATION

Synthesis of the 4'-desmethoxy analogue of RU79115*

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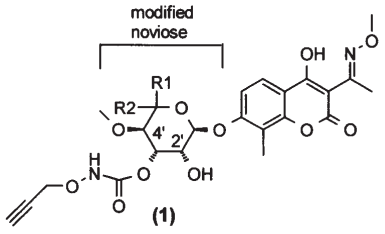
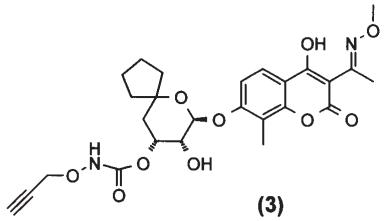
Abstract: The synthesis, and biological activity *in vitro* of the 4'-desmethoxy analogue (**3**) of RU 79115 (**2**) is described. Comparison of the biological activity of the two analogues clearly indicated the importance of the 4'-methoxy group in conferring good gyrase B inhibitory activity as well as antibacterial activity.

Keywords: structure-activity, inhibitor, gyrase B, antibacterial, sugar, L-arabinose, coumarin.

INTRODUCTION

In a previous report from these laboratories[1] the synthesis and structure–activity relationship of a series of coumarin inhibitors (**1**) of DNA gyrase B bearing various 5',5'-dialkylnoviose, and in particular, the most potent derivative RU79115 (**2**) having 5',5'-spirocyclopentyl moiety were described. So far, the role and importance of the 4'-methoxy substituent in the noviose moiety in the binding of coumarin drugs to the active site of gyrase B and its influence regarding antibacterial properties have not been studied. Early crystallographic structures of novobiocin and clorobiocin in a





(2) RU79115, R1=R2= -(CH$_2$)$_4$-

* Dedicated to Professor Živorad Čeković on the occasion of his 70th brithday

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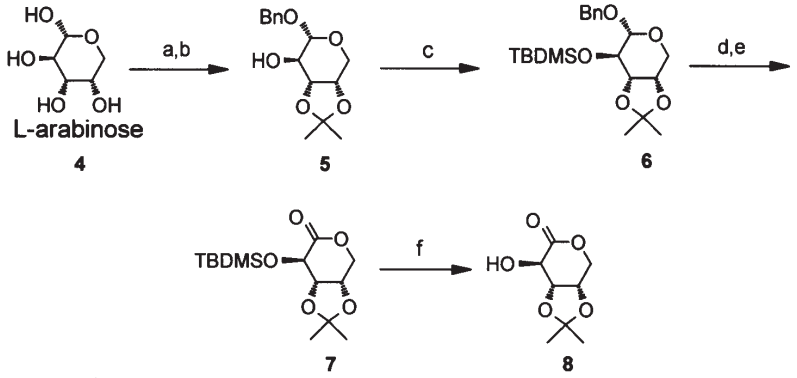
complex with 24 kDa *N*-terminal fragment of gyrase B indicated that the 4'-methoxy group is involved in hydrophobic interactions with the surrounding amino acid residues of the gyrase B protein, as well as in hydrogen bonding to the side chain of Asn-46.[2,3] Further, the 5,5-dimethylcyclohexyl noviose mimic gave some indications regarding the importance of the 4'-methoxy substituent.[4] In order to obtain unambiguous answers to the role of the 4'-methoxy substituent, it was decided to prepare the 4'-desmethoxy analogue of RU79115 and compare directly their activities.

CHEMISTRY

The silyl protected 3,4-*O*-isopropylidene-L-arabino-1,5-lactone (**7**) was chosen as a key intermediate that could provide access to a 4-desmethoxy noviose or to a potential 4-hydroxy and 4-alkyloxynoviose series. So far, two synthetic approaches toward lactone **8** have been described.[5] However, neither of them was suitable for scale-up synthesis. Finally, a five-step synthetic sequence with good overall yield was established (Scheme 1). Starting from L-arabinose (**4**), the corresponding benzyl glycoside was protected as acetonide according to a literature procedure.[6] Silylation of the remaining hydroxyl group under standard conditions provided the fully protected arabinose **6**. Catalytic hydrogenation removed quantitatively the benzyl group and the corresponding lactol was subjected to Swern oxidation to provide the desired silyl-protected lactone **7**. Deprotection of the silyl group was performed under the usual conditions with Bu_4NF to give the hydroxy lactone **8**.



Scheme 1: Reagents and conditions: (a) BnOH, HCl gas, rt, 69 %; (b) 2,2-dimethoxypropane, acetone, TsOH cat, rt, quant; (c) TBDMS-Cl, Im, DMF, rt; (d) H_2, Pd–C/10 %, EtOAc, rt; (e) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, –78 ºC, 75 % from **5**; (f) Bu_4NF, THF, rt, 49 %.

The free hydroxy group of the desilylated lactone **8** (Scheme 2) was then converted to the triflate **9**, which was subjected to reductive conditions with lithium iodide trihydrate[7] to afford the 2-deoxylactone **10** in moderate yield. Lactone opening of **10** with a Grignard reagent of 1,4-dibromobutane provided the diol **11** which was oxidized with two equivalents of $Py{\cdot}SO_3$ directly to the corresponding lac-

tone. This was followed by reduction with DIBALH to provide the lactol **12** which was ready for coupling with the coumarin part. In general, the two-step sequence from diol **11** to lactol **12** gave better yields than the one step oxidation of diol **11** with one equivalent of oxidant. Glycosylation of lactol **12** with 7-hydroxy-8-methyl-4-benzhydryloxycoumarin (**13**)[6] was effected under Mitsunobu conditions in DMF to give exclusively the α-anomer **14**. Deprotection of the benzhydryl group of **14** by catalytic reduction gave the free 4-hydroxycoumarin derivative in quantitative yield. This readily underwent C-acetylation at the 3-position with acetic anhydride and in the presence of DMAP to afford the corresponding coumarin methyl ketone **15**. The acetonide of **15** was easily deprotected with trifluoroacetic acid/water and the diol was subsequently converted to the carbonate **17** with 1,1'-carbonyldiimidazole. The predominantly regioselective opening of the carbonate with *O*-propargylhydroxylamine in the presence of lithium trifluoromethanesulfonate and accompanied with transoximation of the keto functionality afforded a regiomeric mixture of 3'- and 2'-*N*-propargyloxycarbamates in the proportion 3.5:1. This mixture was not separated but was subjected once again to transoximation at the 3-acetyl group with an excess of *O*-methyl hydroxylamine in ethanol. Finally, the 4'-desmethoxy analogue of RU79115 (**2**) was separated from its 2'-*N*-propargyloxycarbamate regioisomer by chromatography on silica gel utilising a mixture CH_2Cl_2-EtOAc-AcOH in the ratio 80:20:1 as eluent.

BIOLOGICAL RESULTS

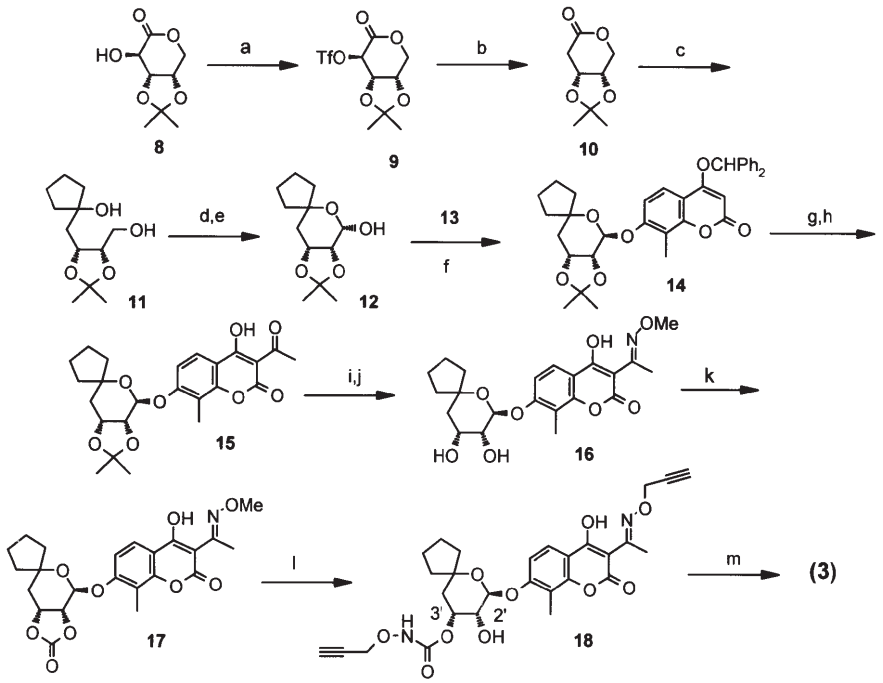
Table I shows the inhibition in the supercoiling activity of *S. aureus* DNA gyrase by novobiocin, RU79115 (**2**) and the corresponding 4'-desmethoxy analogue **3**. Clearly, the absence of the 4'- methoxy group in the noviose part leads to loss of inhibitory DNA gyrase B supercoiling activity and to a loss of the antibacterial properties by two orders of magnitude. Not only the hydrophobic/hydrogen bonding interactions of 4'-methoxy group with the surrounding amino acid residues of gyrase B protein are important in supercoiling inhibition of DNA gyrase, but also the 4'-methoxy substituent plays an important role in intracellular uptake of the coumarin analogues.

TABLE I. *In vitro* inhibitory activity of **2** and **3** against *S. aureus* DNA gyrase B supercoiling activity (IC_{50}),[a] and selected *in vitro* antibacterial activity (MIC).[b]

Compound	Novobiocin	**2**	**3**
IC_{50}nov[a]/IC_{50}comp	1	2.6	0.33
MIC[b] *S. aureus* 011HT3	≤ 0.04	≤ 0.04	1.2

a) IC_{50} was determined for gyrase B of *S. aureus* against novobiocin (0.5 μg/mL) as reference. For the details see Ref. 6; b) MIC, Minimal Inhibitory Concentrations (μg/mL) were measured by using a twofold broth microdilution after 24 h incubation.

In conclusion a synthetic route that leads to 4'-desmethoxy derivatives of noviose analogues has been developed. Silyl lactone **7** could also be a useful inter-



Scheme 2. Reagents and conditions: (a) Tf_2O, CH_2Cl_2, Py, 0 ºC, 89 %; (b) $LiI{\cdot}3H_2O$, THF, AcOH, 41 %; (c) $BrMg–(CH_2)_4–MgBr$, THF, 0 ºC to rt, 54 %; (d) $Py{\cdot}SO_3$, DMSO, TEA, CH_2Cl_2, 63 %; (e) DIBALH, THF, –78 ºC, quant; (f) 7-hydroxy-8-methyl-4-benzhydryloxycoumarin (**13**), PPh_3, $EtO_2CN{=}NCO_2Et$, DMF, rt, 63 %; (g) H_2, Pd–C/10 %, THF, rt, 85 %; (h) Ac_2O, DMAP, CH_2Cl_2, 0 ºC, 82 %; (i) $MeONH_2{\cdot}HCl$, KOAc, EtOH, rt., 65 %; (j) TFA–H_2O 9:1, 84 %; (k) Im_2CO, THF, reflux, 52 %; (l) $HC{\equiv}CCH_2ONH_2{\cdot}HCl$, CF_3SO_3Li, Py, rt, 89 %; (m) $MeONH_2{\cdot}HCl$, KOAc, EtOH, rt., 90 %.

mediate allowing access to 4'-hydroxy or 4'-*O*-alkyls substituted noviose series. Furthermore, future design of novobiocin type inhibitors possessing noviose or noviose mimics should include the 4'-methoxy group or the corresponding hydrophobic isostere in the noviose part in order to confer good antibacterial properties of the analogues.

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ИЗВОД

СИНТЕЗА 4´-ДЕМЕТОКСИ-АНАЛОГА RU79115

БРАНИСЛАВ МУШИЦКИ, АНА-МАРИЈА ПЕРИЈЕ, НИКОЛ ТЕСО, МИШЕЛ КЛИШ

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У раду је описана синтеза и биолошка активност 4´-деметокси аналога (**3**) једињења RU 79115 (**2**). Упоређивање биолошке активности ова два једињења јасно указује на важност и утицај 4´-метокси групе у погледу њихове инхибиторске активности као и антибактеријске активности.

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