Preparation of (5*S*)-5-*C*-methyl-β-L-*lyxo*-hexofuranose derivative as (+)-lactacystin precursors

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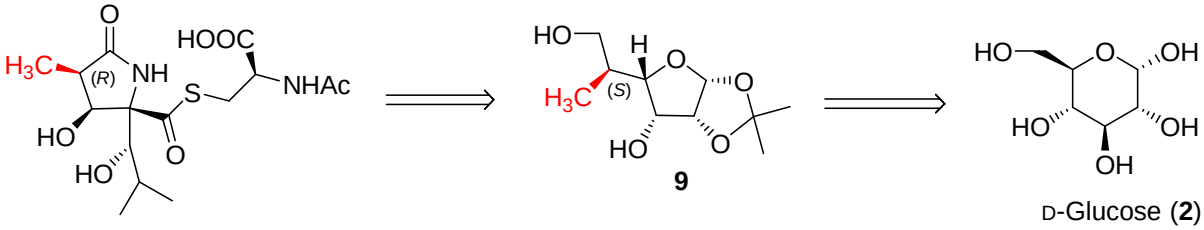
Abstract: *. We present here the preparation of the precursor for stereoselective synthesis of (+)-lactacystin starting from D-glucose. The first step is conversion of D-glucose to 1,2:5,6-di-O-isopropylidene-α-D-gulofuranose. The second step is introduction of a methyl group at C5 and preparation of compound* ***9****. This compound can be used as a precursor of target molecule of (+)-lactacystin .*

Keywords: *Lactacystin, Asymetric synthesis, Proteasome inhibitor*

1. Introduction

(+)-Lactacystin (**1**) is a secondary metabolite derived from a streptomyces bacterial strain (OM-6519) found in a Japanese soil sample. The structure and absolute configuration of the natural product was determined by NMR spectroscopy and an X-ray crystallographic analysis. Further interpretation of the biological activity of **1** was to come with the discovery by Fentaeny and co-workers of the molecular target, the proteasome, which is responsible for intracellular protein degradation.[1] (+)-Lactacystin (**1**) was the first identified natural 20S proteasome inhibitor. It selectively binds to the *β*-oxygen atom of the *N*-terminal threonine in the *β*5 subunit of the 20S proteasome through an ester covalent bond. Because this oxygen atom is crucial for the enzymatic activity, lactacystin is an irreversible inhibitor of the proteasome. Proteasome targeting has recently emerged as a new modality for the potential treatment of diseases ranging from malaria to cancer.[2]

Scheme 1. Our strategy for synthesis of (+)-lactacystin



(+)-Lactacystin (**1**) D-Glucose (**2**)

Corey et al. reported the first synthesis of compound **1** in 1992.[3] He also prepared a number of analogs of **1**. There are many reports of the total synthesis of (+)-lactacystin using a variety of asymmetric reactions such as aldol reaction, Diels-Alder cyclization, but also Overman rearrangement. The key step for Chida synthesis in 1995 was [3.3]-sigmatropic Overman rearrangement to install the quaternary C5 stereocenter with moderate levels of stereoselection. The start compound of this synthesis was D-glucose.[4]

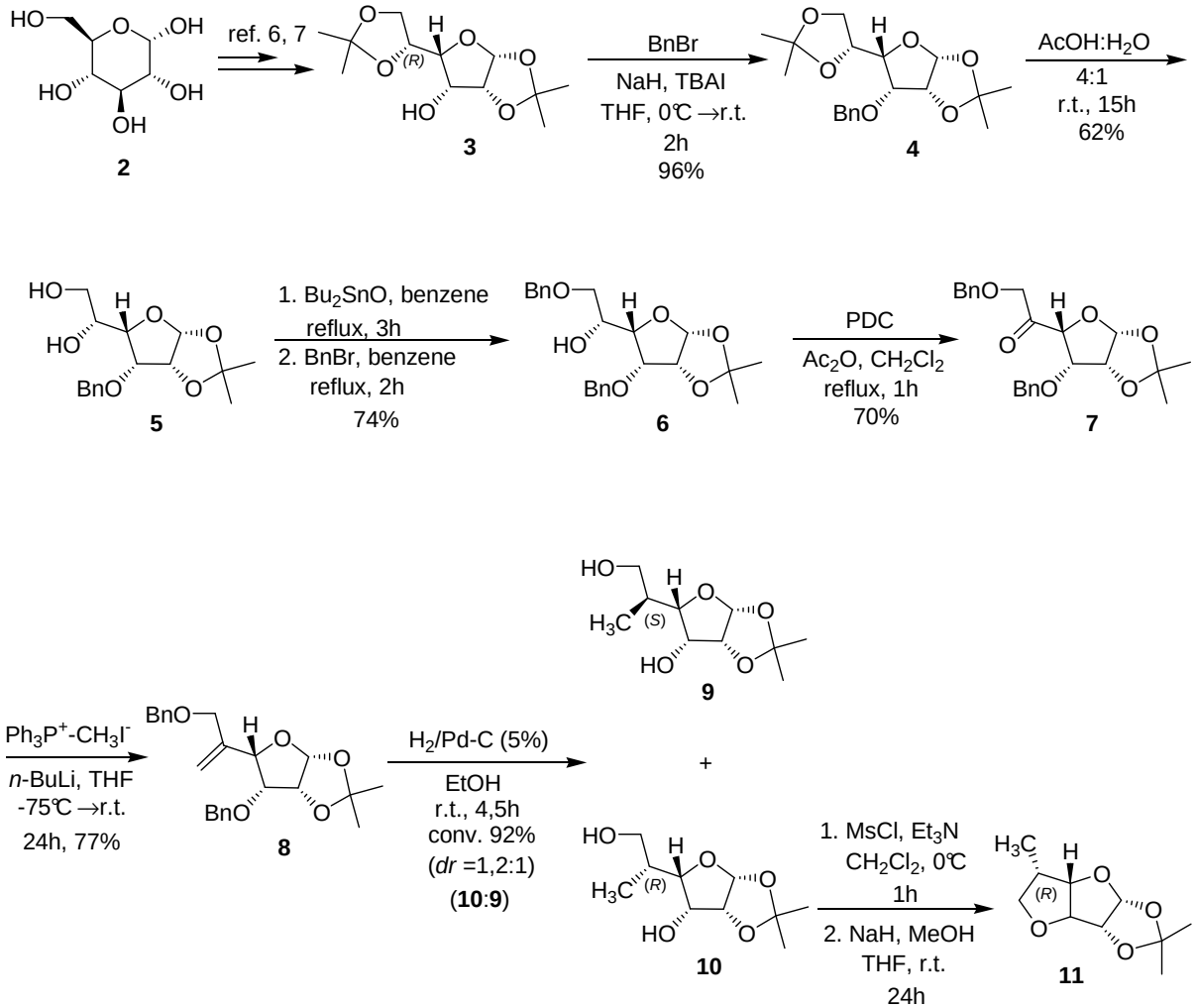
We present here preparation of **9** as precursor for stereoselective synthesis of (+)-lactacystin starting from D-glucose (Scheme 1). Our group is using [3.3]-sigmatropic rearrangements for the synthesis of natural compounds, therefore, we are planning to use

the stereoselective Overman [3.3]-sigmatropic rearrangement also in the synthesis of (+)-lactacystin .[5]

2. Results and discussion

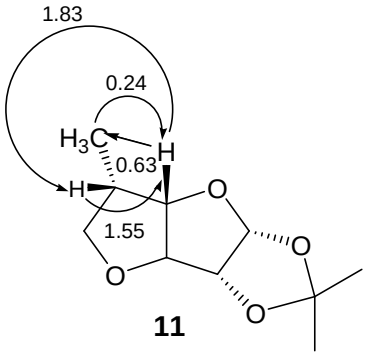
In first steps, 1,2:5,6-di-*O*-isopropylidene-α-D-gulofuranose (**3**) was prepared from D-glucose (**2**).[6,7] The following reaction sequence was performed in order to introduce the methyl group at position C5. Reaction of **3** with sodium hydride in THF using PTC conditions (TBAI) gave the corresponding alkoxide, to which benzyl bromide was added to yield the *O*-benzyl derivative **4**. Next reaction was selective deprotection of one of isopropylidene groups in mixture AcOH/H_2O, resulting in compound **5**. The primary hydroxy group in **5** was selectively benzylated to afford **6** in 74% yield. The secondary hydroxy group in **6** was oxidized using PDC/Ac_2O in CH_2Cl_2 to obtain ketone **7**. The Wittig reaction of **7** with ylide afforded compound **8**. The ylide was generated *in situ* through the reaction of phosphonium salt with the butyllithium. The mixture of diastereoisomers **9** and **10** (*dr* =1,2:1 **10**:**9**) was prepared *via* catalytic hydrogenation of compound **8** at room temperature over 5% Pd-C in 92% conversion (Scheme 2). The diastereoisomeric ratio (*dr* = 1,2:1) was established from 1H NMR spectrum of the mixture of diastereoisomers. We are planning to improve the diastereoisomeric ratio of this reaction using asymetric catalysts.

Scheme 2. The introduction of methyl group at position C5



We assigned the absolute configuration at C5 of compound **10** from NOE experiment performed on compound **11** (Fig. 1). Compound **11** was prepared through mesylation of **10** and subsequent cyclization (Scheme 2).

Figure 1. NOE (%) of compound **11**



3. Experimental

The compound **3** was prepared according to literature.[6,7]

3-*O*-benzyl-1,2:5,6-di-*O*-isopropylidene-α-D-gulofuranose (4): To a stirred solution of **3** (6,1 g, 23,4 mmol) and NaH (786 mg, 32,8 mmol, 1,4 equiv.) in dry THF (135 ml) at 0℃ was added Bu_4NI (864 mg, 2,34 mmol, 0,1 equiv.) After 30min, BnBr (4,8 g, 28,1 mmol, 1,2 equiv.) was added dropwise at 0℃ and after 30 min the reaction mixture was warmed to room temperature. The mixture was stirred at room temperature for 1,5 h under N_2 and monitored by TLC using hexane:EtOAc (1:1, v/v). To a reaction mixture was added MeOH and water, mixture was extracted CH_2Cl_2 (2x). The organic solution was dried over Na_2SO_4, filtered and evaporated. The residue was purified by silica gel column chromatography by using hexane–EtOAC 3:1 as the mobile phase. The fractions corresponding to product were collected and dried. Those yielded 7,9 g of **4** as white solid (96%).

3-*O*-benzyl-1,2-*O*-isopropylidene-α-D-gulofuranose (5): The *O*-benzyl derivative **4** (7,1 g, 20,3 mmol) was dissolved in 57 ml mixture $AcOH/H_2O$ (4:1). The mixture was stirred at room temperature for 15 h and monitored by TLC using hexane:EtOAc (1:1, v/v). To a reaction mixture was added 12,5% water solution NaOH (108 ml) and mixture was extracted EtOAc (2x). The organic solution was dried over Na_2SO_4, filtered and evaporated. The residue was purified by silica gel column chromatography (hexane–EtOAC 1:1) to give **5** (3,9 g, 62%) as a white solid.

3,6-di-*O*-benzyl-1,2-*O*-isopropylidene-α-D-gulofuranose (6): A compound **5** (3,7 g, 11,9 mmol) was refluxed with *n*-Bu_2SnO (3,27 g, 13,1 mmol, 1,1 equiv.) 2,5 h in dry benzene (120 ml) with continuous removal of water in a Dean-Stark apparatus. The solvent was evaporated and residue was dissolved in dry benzene (53 ml), Bu_4NI (4,4 g,11,9 mmol, 1 equiv) and BnBr (2,12 g, 12,4 mmol, 1,04 equiv.) was added at room temperature. The reaction mixture was stirred and refluxed for 2 h under N_2 and monitored by TLC using hexane:EtOAc (1:1, v/v). The solvent was evaporated and the residue was purified by silica gel column chromatography (hexane–EtOAC 3:1) to give **6** (3,5 g, 74%) as a colorless oil

3,6-di-*O*-benzyl-1,2-*O*-isopropylidene-β-L-*lyxo*-hexofuranos-5-ulose (7): The compound **6** (2,64 g, 6,6 mmol) was dissolved in dry CH_2Cl_2 (21 ml) along with Ac_2O (1,76 ml). PDC (1,74 g, 4,6 mmol, 0,7 equiv.) was added to the reaction which was refluxed 6 h under N_2 and monitored by TLC using hexane:EtOAc (3:1, v/v). The solvent was evaporated and

residue was washed with Et_2O, filtered and solvent was evaporated (2x). The mixture was purified by silica gel column chromatography (hexane–EtOAc 3:1) to give **7** (1,77 g, 67%) as a colorless oil.

3,6-di-*O*-benzyl-5-deoxy-1,2-*O*-isopropylidene-5-*C*-methylene-β-L-*lyxo*-hexofuranose (8): The methylenation reagent was prepared by addition of 1,6M butyllithium-hexane solution (0,71ml, 1,13 mmol, 3 equiv.) to a suspension of methyltriphenylphosphonium iodide (456 mg, 1,13 mmol, 3 equiv.) in dry THF (2 ml) at -20℃ under N_2. The yellow solution was stirred for 10min at -20℃, warmed to room temperature, and stirred for 25 min, cooled to -70℃, and a solution of ketone **7** (150 mg, 0,38 mmol) in dry tetrahydrofuran (4 ml) was added dropwise. The mixture was stirred for 1 h at -70℃ and then for 22,5 h at room temperature under N_2 and monitored by TLC using hexane:EtOAc (3:1, v/v). Water was added, and the mixture was extracted with diethyl ether. Drying (Na_2SO_4) and customary processing gave a residue which was purified by silica gel column chromatography (hexane–EtOAc 9:1), to afford alkene **8** as a colorless oil (114,7 g, 77%).

(5*S*)-5-deoxy-1,2-*O*-isopropylidene-5-*C*-methyl-β-L-*lyxo*-hexofuranose (9)
(5*R*)-5-deoxy-1,2-*O*-isopropylidene-5-*C*-methyl-β-L-*lyxo*-hexofuranose (10): The alkene **8** (475 mg, 1,2 mmol) was dissolved in dry ethanol (10,3 ml) and hydrogenated at room temperature over 5% Pd-C (300 mg). The reaction was monitored by TLC using hexane:EtOAc (3:1, v/v). After 4,5 h, the catalyst was removed by filtration and the solvent was removed by evaporation. The conversion of reaction was 92% (61 mg) and mixture of diastereoisomers (1,2:1) was purified by silica gel column chromatography (CH_2Cl_2-MeOH 70:1), to afford derivative **9** and **10** as a colorless oils.

3,6-anhydro-1,2-*O*-isopropylidene-5-*O*-(methyl)-α-D-gulofuranose (11): To a stirred solution of **10** (70 mg, 0,32 mmol) in dry CH_2Cl_2 (2,8 ml) at 0℃ was added Et_3N (42 mg, 0,42 mmol, 1,3 equiv.) and MsCl (44 mg, 0,39 mmol, 1,2 equiv.) The mixture was stirred at 0℃ for 1 h under N_2 and monitored by TLC using hexane:EtOAc (1:1, v/v). The solvent was evaporated and residue was washed with Et_2O, filtered and solvent was evaporated. The product was dissolved in dry THF (1,8 ml), NaH (23 mg, 0,48 mmol, 1,5 equiv.) and MeOH (0,5 ml) was added. The mixture was stirred at room temperature for 48 h under N_2 and monitored by TLC using hexane:EtOAc (1:1, v/v). The reaction mixture was neutralized with acetic acid and extracted CH_2Cl_2 (2x). Drying (Na_2SO_4) and customary processing gave a residue which was purified by silica gel column chromatography (hexane–EtOAc 1:1), to afford **11** as a colorless oil (25 mg, 39%).

NMR spectra were consistent with structures.

4. Conclusion

We have synthesized a new compound **9** which is a precursor for stereoselective synthesis of target molecule (+)-lactacystin. The number of synthetic steps was 7. Our further plan is to use [3.3]-sigmatropic Overman rearrangement to prepare the trichloroacetamide and complete the synthesis of the target molecule (+)-lactacystin (**1**).

5. Acknowledgements

We are gratefully acknowledging the financial support provided by the Grant Agency (No. 1/0100/09 and 1/0281/08) of the Ministry of Education of Slovak republic, Research and development support agency, Slovak republic VVGS PF 6/2010/Ch.

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