Available online at www.sciencedirect.com
SCIENCE DIRECT
Carbohydrate Research 341 (2006) 433–442
Carbohydrate RESEARCH

Synthesis, structural studies, and cytostatic evaluation of 5,6-di-*O*-modified L-ascorbic acid derivatives

Tatjana Gazivoda,[a] Karlo Wittine,[a] Iva Lovrić,[a] Damjan Makuc,[b] Janez Plavec,[b] Mario Cetina,[c] Draginja Mrvoš-Sermek,[d] Lidija Šuman,[e] Marijeta Kralj,[e] Krešimir Pavelić,[e] Mladen Mintas[a] and Silvana Raić-Malić[a,*]

[a]*Department of Organic Chemistry, Faculty of Chemical Engineering and Technology, University of Zagreb, Marulićev trg 19, HR-10000 Zagreb, Croatia*
[b]*Slovenian NMR Centre, National Institute of Chemistry, Hajdrihova 19, PO Box 660, SL-1001 Ljubljana, Slovenia*
[c]*Faculty of Textile Technology, University of Zagreb, Pierottijeva 6, HR-10000 Zagreb, Croatia*
[d]*Laboratory of General and Inorganic Chemistry, Faculty of Science, University of Zagreb, Zvonimirova 8, HR-10000 Zagreb, Croatia*
[e]*Division of Molecular Medicine, Ruđer Bošković Institute, Bijenička 54, HR-10000 Zagreb, Croatia*

Received 7 November 2005; received in revised form 23 December 2005; accepted 24 December 2005
Available online 24 January 2006

Abstract—The 5,6-di-*O*-tosylated derivative of L-ascorbic acid was synthesized by selective protection and deprotection of 2,3- and 5,6-dihydroxy functional groups involving 5,6-ditosylation in the final step, while the novel 6-acetoxy, 6-hydroxy, and 6-chloro derivatives of 4,5-didehydro-L-ascorbic acid were obtained by reaction of ditosylated compound with nucleophilic reagents. The analysis of $^{3}J_{\text{H-4–H-5}}$ homonuclear coupling constants shows that all L-ascorbic acid derivatives except for epoxy and 4,5-didehydro compounds exist in high population as gauche conformers across C-4–C-5 bonds, while $^{3}J_{\text{C-3–H-5}}$ heteronuclear coupling constants in 4,5-didehydro derivatives indicate cis geometry along C-4–C-5 double bond. The X-ray crystal structure analysis of 2,3-di-*O*-benzyl-5,6-epoxy- and 5,6-isopropylidene-L-ascorbic acid shows that the oxygen atoms attached at positions 2 and 3 of the lactone ring are disposed in a *synperiplanar* fashion. Besides that, the dioxolane ring adopts half-chair conformation. The molecules of epoxy derivative are joined into infinite chains by one weak hydrogen bond of C–H···O type. Two O–H···O, and C–H···O hydrogen bonds link the molecules of 5,6-di-*O*-isopropylidene compound into two-dimensional network. 6-Chloro derivative of 2,3-di-*O*-benzyl-L-ascorbic acid showed the best cytostatic effects against all tested malignant tumor cells (IC_{50}: ~18 μM).

Keywords: L-Ascorbic acid; Configurational analysis; Conformational analysis; Single crystal X-ray diffraction; Cytostatic activity

1. Introduction

L-Ascorbic acid (vitamin C) is the trivial name for the six-carbon sugar derivative L-*threo*-hex-2-enono-1,4-lactone. It is one of the most important biomolecule, which acts as antioxidant and radical scavenger, and is widely distributed in aerobic organisms. Thus, it protects cellular compounds against oxidative damage by free radicals and oxidants.[1] L-Ascorbic acid is present in all foods of plant origin and is an essential micronutrient in man as a consequence of the absence of L-gulonolactone oxidase.[2] It is also crucial for the biosynthesis of collagen, which is a main component of dentin, bone, connective tissue, etc.[3] Furthermore, the biological importance of L-ascorbic acid, known as a putative palliative against the common cold, was initially associated with scurvy, the symptoms of vitamin deficiency.[4] The well-known susceptibility of vitamin C to thermal and oxidative degradation has led to interest in L-ascorbic acid derivatives with increased stability. Numerous simple derivatives of L-ascorbic acid have been synthesized and shown to possess important pharmacological properties. For example, 5,6-di-*O*-modified ascorbic acid

* Corresponding author. Tel.: +385 1 4597 214; fax: +385 1 4597 250; e-mail: silvana.raic@pierre.fkit.hr

0008-6215/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carres.2005.12.010

derivatives were clinically affective antitumor agents for various human cancers[5] and also induced apoptosis in tumor cells,[6] 2-*C*-alkylated derivatives have been shown to have immunostimulant activity,[7] and 2-*O* and 3-*O*-alkylated lipid soluble derivatives are known to protect against the lipid peroxidation of the biomembrane.[8] Some pyrimidine and purine derivatives of 4,5-didehydro-5,6-dideoxy-L-ascorbic acid exerted pronounced cytostatic activities against malignant tumor cell lines.[9]

The present study deals with the synthesis of novel 5,6-di-*O*-modified 4,5-didehydro-L-ascorbic acid derivatives, the configurational and conformational analysis using $^{1}H/^{13}C$ NMR spectroscopy, X-ray crystallography, and the evaluation of their antitumoral potencies.

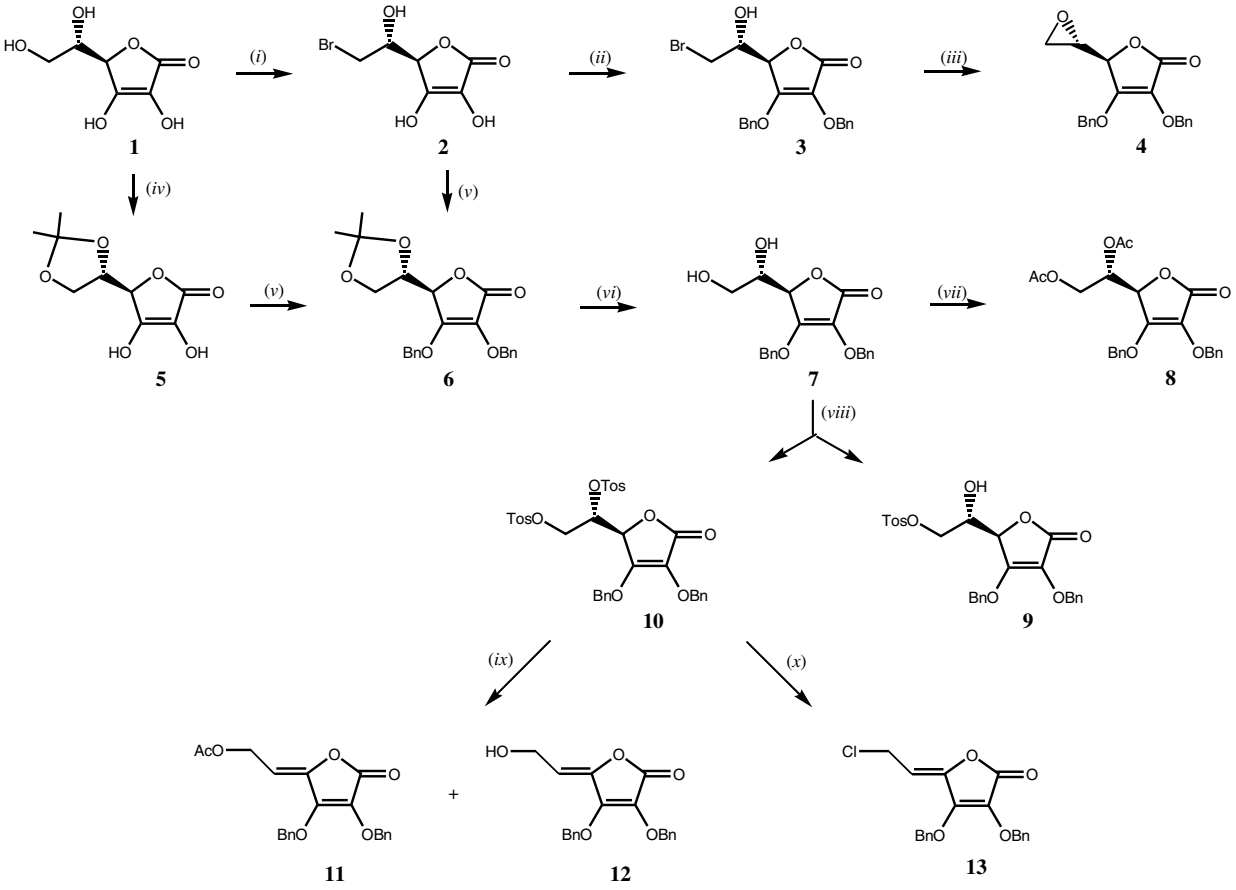
2. Results and discussion

2.1. Compounds preparation

The 5,6-epoxy **4**[10] and 5,6-di-*O*-acetyl-2,3-di-*O*-benzyl-L-ascorbic acid **8**[10] were synthesized from the starting L-ascorbic acid as outlined in Scheme 1. The 5,6-di-*O*-tosylate derivative of L-ascorbic acid **10** was prepared by a sequence of reactions involving selective protection of 5,6-dihydroxy groups in **7**[11] using toluene-4-sulfonyl chloride. Reaction of the ditosylated compound **10** with sodium acetate gave both, 6-acetoxy **11** and 6-hydroxy **12** derivatives of 4,5-didehydro-L-ascorbic acid. The 6-chloro derivative of 2,3-di-*O*-benzyl-L-ascorbic acid **13** was prepared by reaction of ditosylate **10** with diazobicycloundecane and tetraethylammonium chloride. These reactions of the ditosylated compound **10** most likely involve a two-step reaction pathway in which the first step is an E2 elimination reaction that produces an exocyclic allylic tosylate, which then undergoes an S_N2 reaction with the corresponding nucleophile to give **11** and **13** and in the next step the hydrolysis product **12**. Compounds **11**–**13** were found to exist as *Z*-isomers.

2.2. Configurational and conformational assessment by ^{1}H and ^{13}C NMR

The assignments of ^{1}H and ^{13}C NMR spectra of **2**–**13** are reported in Tables 1 and 2, respectively. *C*-Methyl



Scheme 1. Reagents: (i) HBr/CH_3COOH; (ii) benzyl chloride, K_2CO_3; (iii) satd aq solution Na_2CO_3; (iv) acetone, acetyl chloride; (v) benzyl chloride, K_2CO_3; (vi) 50% CH_3OOH; (vii) acetic acid anhydride, pyridine; (viii) toluene-4-sulfonyl chloride, pyridine; (ix) NaOAc, DMF; (x) DBU, Et_4NCl.