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January 27, 2014

## REVIEW OF THE DOCTORATE DISSERTATION

Doctoral thesis entitled "Biomimetic carbon-carbon bond formation: synthesis of ulosonic acids"

Presented by Mr. Osama El-Sepelgy

Prepared under the supervision of Professor Jacek Młynarski

Submitted to the Scientific Council of Faculty of Chemistry, Jagiellonian University in Cracow, Poland

### Introduction

Ulosonic and sialic acids are monosaccharide derivatives structurally representing the products of oxidation of the 1-hydroxyl group of a ketose, thus creating the corresponding  $\alpha$ -ketoacid. The six- to nine-carbon compounds are formed in the aldolase-catalyzed addition of the three carbon unit (usually phosphorylated enol of pyruvate) to glyceraldehyde, erythrose, pentose or hexose, such as KDO (3-deoxy-D-manno-oct-2-ulosonic acid). These compounds are used in the synthesis of lipopolysaccharides forming the bacteria cell walls. For the significant biological roles derivatives of ulosonic acids are important targets for the synthesis of currently used or prospective pharmaceuticals.

Biosynthesis of ulosonic acids involves catalyzed stereoselective aldol reaction. There are two different classes of aldolase. Aldolase I catalyzes transfer of three-carbon ketose units *via* a Schiff base intermediate, whereas aldolase class II uses a zinc cation as a co-factor, offering an electrostatic stabilization of a negative charge on the keto oxygen of the substrate. As two classes of aldolase catalyze the reaction in nature, analogously two types of designed catalysts have been applied "in a flask". One of them are metal-based catalysts, mostly activating the electrophilic character of a carbonyl carbon atom.

The main aim of the research described in the dissertation was elaboration of the synthetically useful stereoselective aldol reaction leading to the title ulosonic acids. The declared biomimetic approach suggested that both metallo- and organocatalysts will be studied in order to achieve this uneasy goal. The topic closely corresponds to the well-known chemistry fruitfully developed by the Applicant's mentor, Prof. Jacek Młynarski.

### Review of the Applicant's Scientific Achievements

After a general introduction focusing the dissertation topic in the field of natural science, the essentials of chemistry of ulosonic acids are shortly presented. This consistent chapter is well written and adequate to the main topic. It describes a biosynthesis and four different approaches to the chemical synthesis of the title acids. A review of the relevant literature shows that, in spite of some

previous efforts, the successful stereoselective cross-aldol reaction of pyruvates has been reported for just a few non-enolizing, highly active substrates. Moreover, the other attempted catalyzed aldol reactions of pyruvates resulted in the homo-aldol additions only. Thus, I assess the synthetic goal undertaken by Mr. El-Sepelgy as being really challenging.

The references cited within this chapter are appropriate and quite recent (together 88 papers, more than half of them were published in this century) The presented review is in my opinion informative and enough comprehensive.

The main body of the own research presented in the dissertation is described in two already published papers: (i) "Direct Aldol Reaction of Pyruvic Derivatives: Catalytic Attempt to Synthesize Ulosonic Acids" (O. El-Sepelgy, D. Schwarzer, P. Oskwarek, J. Młynarski, *Eur. J. Org. Chem.* **2012**, 2724-2727.) and (ii) "Biomimetic Direct Aldol Reaction of Pyruvate Esters with Chiral Aldehydes" (O. El-Sepelgy, J. Młynarski, *Adv. Synth. Catal.* **2013**, 355, 281-286.). Experimental details, spectra, etc. of the work reported in the papers are placed in the Supporting Information Parts, that were also published and are available on the respective Internet pages. Both scientific journals (publisher Wiley-VCH) are highly respected in the scientific community and enjoy wide readership. Their 2012 IF's are 3.344 and 5.535, respectively. As it is required by the regulations (see below), the Applicant's crucial role in the published work is clearly confirmed by prof. J. Młynarski. The two remaining co-authors of the first paper were undergraduate students and, as it is indicated by prof. J. Młynarski, they were guided by Mr. El-Sepelgy.

In the first paper the asymmetric aldol reaction of pyruvic aldehyde dimethyl acetal and 2-acetylthiazole (Dondoni's masked pyruvate) with chiral aldehydes is reported. The reactions were catalyzed by zinc-based chiral Trost and the Lanthanum-Lithium-Binol-Shibasaki catalysts. Discovery of the effective catalytic systems for the efficient and stereoselective aldol reaction of pyruvate - sugar aldehydes can be regarded as a really meaningful achievement in the synthesis of 3-deoxy-2-ulosonic acids.

Nonenzymatic systems that reproduce in some measure the constituents of the active site of an enzyme are often branded as biomimetic. In this case, the Authors come to their discovery by reasoning that follows to some extent the known activity of aldolase II. At first, they carried out a reference reaction between glyceraldehyde and lithium enolate of pyruvic aldehyde dimethyl acetal and after 20 min. they got 35% of the desired product. Then, they tested in this reaction both mentioned chiral metal-based catalysts getting the substantial improvement in terms of yield/dr. Turning to the Dondoni intermediate, in the reaction catalyzed by 5 mol% of (S)-LLB, they obtained the respective KDG precursor in 53% yield and 75% de. The same intermediate was used in the reaction catalyzed by 5 mol% of the Trost chiral complex of zinc affording 77 %, 60 % de of aldol, precursor of KDO. Indeed, the outcome of this work can be considered as an important breakthrough in the synthesis of ulosonic acids.

The second paper reports direct aldol reactions of sterically hindered aryl pyruvate esters with sugar aldehydes promoted by the same as previous dinuclear metal complexes as well as chiral *Cinchona* alkaloid organocatalysts. Application of 2,6-di-tert-butyl-4-methoxyphenyl pyruvate ester allowed for the aldol reaction of diacetonide of glyceraldehyde with *syn*- or *anti*-selectivity, depending on the configuration of the catalyst. Thus, 6'-isopropoxy-derivative of cinchonidine gave the corresponding *anti*-adduct (80 %, 88 % de), while the use of the same derivative of quinidine resulted in low excess of the *syn*-product. The reaction with cinchonidine-catalyst led to the (S)-configuration at the newly created center, regardless the configuration of sugar-aldehyde. Accordingly, the reaction of arabinose diacetonide gave the *syn*-adduct (60 %, 78 % de) that could be easily transformed into 4-

*epi*-KDO, the product of pharmaceutical importance. The observed stereochemical reaction pattern was summarized in a sketch of the proposed substrates-catalyst complex, leading to the *Si*-face addition (Scheme 5, p. 284). The postulated mechanism of the catalyst participation seems quite reasonable, regardless it is far from that of aldolase I (Schiff base as an intermediate). In my opinion, the synthetic method using *Cinchona* alkaloid catalysts mimics aldolase-catalyzed reaction in a very general sense only (organocatalyst operating in the absence of metal-ions). On the other hand, the findings are of essential significance. The Authors report efficient method for the practical short synthesis of important pharmaceutical products avoiding the introduction of any metal-impurities. Moreover, the catalyzed reaction leads to the stereodivergent products and the outcome can be controlled by the use of proper catalyst and the catalysts are quite affordable. I am sure, the reported results will enjoy much interest worldwide. It is also noteworthy that the results reported in the second paper have arisen as a smart development of the previous discoveries, described in the first paper. An ambitious target set as the aim of the research has been reached and the new convenient synthesis of ulosonic acids was developed.

The doctorate dissertation is supplemented with a reprint of a book chapter entitled "Aldol Reaction", taken from a volume *Science of Synthesis, Water in Organic Synthesis*, Shu Kobayashi Ed., Georg Thieme Verlag, 2012, pp. 353-384 which is authored by M. Woyciechowska, O. El-Sepelgy, and J. Młynarski. Even if this published work is not presented as a result of study for the degree, it clearly documents the scientific competence of the Applicant.

Moreover, Mr. El-Sepelgy enriched his studies on the C-C bond forming aldol reaction with an interesting work on the conjugate addition of different C-nucleophiles to the *in situ* generated *ortho*-quinomethide chiral intermediates. This paper reports his research conducted at the laboratory of prof. C. Schneider, Institut für Organische Chemie, Universität Leipzig, Germany. It has not been published in the scientific journal as yet. However, as it reports an original experimental work, I consider it as an additional proof of the Applicant's scientific competence. Mr. El-Sepelgy successfully applied phosphoric acid esters of modified Binol as a chiral Brønsted acid catalyst in a highly enantioselective synthesis of chromones. The stereochemical outcome of the reaction was proved by the single-crystal X-ray structure determination of the product. Finally, the dual activated transition state for the enol addition to *o*-quinomethide was proposed. All this section confirms high qualifications and abilities of the Applicant.

Except for the already printed materials, in some instances the English used needs careful attention. Also some editorial amendments are advisable before further publication. Was dr. Cossi (?) really involved in this research (as it is written, p. 28, and also p. 1 of the paper on conjugate addition) or prof. Pier Giorgio Cozzi (not P.C. Cozzi as in ref. 85 and ref. 5) should be rather mentioned there? The minor shortcomings do not diminish the scientific quality of the thesis, which I rate very high.

### Final Remarks

Doctorate dissertation "Biomimetic carbon-carbon bond formation: synthesis of ulosonic acids" prepared by Mr. Osama El-Sepelgy is an original scientific work. The Author of this work proved to be a fully qualified researcher able to plan and conduct experiments as well as describe the obtained results of scientific research. The range of presented experimental work and structural analysis is wide enough, and the main parts of the results have already been published in the highly respected,

international journals. The obtained results are scientifically sound and the already published papers surely will draw attention from many researchers worldwide.

In conclusion, the reviewed dissertation completely fulfils all the requirements of the Polish regulations concerning academic degrees as defined in the Polish Code of Scientific Degrees and Title of 14 March 2003 with later amendments, (Article 13, Sections 2, 4, and 5).

In my opinion, Mr. Osama El-Sepelgy deserves for admission to the next stages of the doctorate-granting procedure.

Taking into account novelty and the range of research I recommend this dissertation for its consideration as a candidate for an appropriate award.

A handwritten signature in black ink, appearing to be 'A. J. J.' followed by a long horizontal stroke.