Chemoselective Oxidation of Isoxazolidines with Ruthenium Tetroxide: A Successful Intertwining of Combined Theoretical and Experimental Data

Laura Legnani 1, Salvatore V. Giofré 2, Daniela Iannazzo 3, Consuelo Celesti 3,4, Lucia Veltri 5 and Maria Assunta Chiacchio 6,*

1 Dipartimento di Biotecnologie e Bioscienze, Università di Milano-Bicocca, Piazza della Scienza 2, 20126 Milano, Italy
2 Dipartimento di Scienze Chimiche, Biologiche, Farmaceutiche ed Ambientali, Università di Messina, Viale F. Stagno D’Alcontres, 98166 Messina, Italy
3 Dipartimento di Ingegneria, Università di Messina, Contrada di Dio, 98166 Messina, Italy
4 Dipartimento di Medicina Clinica e Sperimentale, Università di Messina, Via Consolare Valeria, 98125 Messina, Italy
5 Dipartimento di Chimica e Tecnologie Chimiche, Università della Calabria, Via Pietro Bucci 12/C, 87036 Aracacavata di Rende, Italy
6 Dipartimento di Scienze del Farmaco e della Salute, Università di Catania, Viale A. Doria 6, 95125 Catania, Italy
* Correspondence: ma.chiacchio@unict.it

Abstract: The direct oxidation reaction of isoxazolidines plays an important role in organic chemistry, leading to the synthesis of biologically active compounds. In this paper, we report a computational mechanistic study of RuO₄-catalyzed oxidation of differently N-substituted isoxazolidines 1a-c. Attention was focused on the endo/exo oxidation selectivity. For all the investigated compounds, the exo attack is preferred to the endo one, showing exo percentages growing in parallel with the stability order of transient carbocations found along the reaction pathway. The study has been supported by experimental data that nicely confirm the modeling results.

Keywords: ruthenium tetroxide; oxidation; DFT calculations; 3-isoxazolidinone; chemoselectivity

1. Introduction

Heterocyclic chemistry [1,2] represents one of the most complex and fascinating branches of organic chemistry of equal interest for its theoretical implications [3,4], involving also almost all aspects of modern organic chemistry. The synthesis and functionalization of heterocycles hold a pivotal role in medicinal chemistry, showing a wide range of pharmaceutical and biological properties. Moreover, heterocyclic compounds are key elements in vitamins, hormones, alkaloids, herbicides, dyes, and other products of industrial importance [5–7]. Among the different classes of heterocyclic compounds, some isoxazolidine derivatives, analogs of natural nucleosides and nucleotides, have shown great interest for their anticancer and antiviral properties [8–11]. The synthetic strategies towards these five-membered heterocyclic rings mostly exploited are the classical 1,3-dipolar cycloadditions of nitrones with differently substituted dipolarophiles [12]. The so-formed cycloadducts can be furtherly functionalized to give the 3-isoxazolidinone nucleus, a cyclic Weinreb amide, whose reduction [13] and nucleosidation lead to reverse transcriptase inhibitors [14]. The first example of direct oxidation of isoxazolidines to the 3-isoxazolidinones was reported in the literature in 2007 [15]. This transformation carried out using RuO₂/NaIO₄, under ethyl acetate/water biphasic conditions, proved to be highly regioselective, giving only 3-isoxazolidinone derivatives as exclusive compounds.

Based on our expertise in the field of computational mechanistic studies [16,17], we have performed a preliminary study of ruthenium tetroxide-mediated oxidation of
some cyclic and heterocyclic compounds. In these studies, DFT and topological methods highlighted that, on these substrates, the rate-limiting step of the reaction takes place through a highly asynchronous (3 + 2) concerted cycloaddition [18]. More recently, we reported a complete computational mechanistic study concerning the oxidation reaction of 2-methylisoxazolidine with RuO₄, taking into consideration the different sites where the oxidation could take place [19]. In fact, all the hydrogen atoms of the isoxazolidine system (C-1, C-3, C-5, and C-4) could be transferred by oxidation with RuO₄ and reactions appeared to be competitive. However, the corresponding barriers for oxidation resulted to be correlated to the stability of the transient carbocation forming along the reaction pathway. So, the N-methylisoxazolidin-3-one was detected as the preferred product.

In this paper, with the support of experimental data, we have extended our computational study to isoxazolidines 1a–c (Figure 1), bearing a methyl-carboxylate group at C-5 of the isoxazolidine ring, to avoid competition with oxidation in this position [19]. The results of this study will correlate with the endo/exo oxidation selectivity and follow the carbocations stability order.

![Figure 1. N-substituted isoxazolidines.](image)

2. Results and Discussion

2.1. Computational Investigation

All calculations were performed using the Gaussian16 program package [20]. After a preliminary screening considering different levels of calculation already tested on analogous systems, as reported in the literature [18,19], optimizations were performed using the B3LYP functional [21,22] in conjunction with Grimme’s dispersion correction [23,24] (henceforth referred to as B3LYP-d3bj) chosen referring to similar systems’ studies [18,19]. The standard basis set Def2SVP was employed [25,26]. Solvent effects water, using the PCM method [27,28] were taken into consideration. As reported [19], the highly asynchronous (3 + 2) one-step oxidation mechanism [29–34] presents two different stages: (a) activation of the R-CH bond, coordinated to the Ru(VI); (b) transfer of the second hydrogen with the obtainment of the oxygenated compound and Ru(IV) (Scheme 1). The first stage is the regioselectivity-determining one and was firstly computationally investigated.

In all the cases, the transition states (TS1), leading to P1 for both the endo and exo pathways (Scheme 1), were located and their 3D plots are reported in Figure 2. For the exo pathway, due to the presence of the stereogenic center at position 5 of the isoxazolidine, and the formation of a second stereogenic center for compounds 1a and 1c, the two possible pro-R and pro-S transition states have been considered. Conversely, for the endo route, it was not possible to locate the pro-S transition state, due to the steric hindrance caused by the presence on C-5 of the methylcarboxylate moiety. For the N-cyclohexyl derivative 1b, the two possible 1C₄ and 4C₁ chair conformations of the six-membered ring, have been examined. The percentages of the compounds derived from the TSs at 298 K, were calculated and the corresponding values are given in Table 1. As expected, contrary to the endo preference detected for the N-methylisoxazolidine [19], in all the studied compounds, the exo adduct resulted in being favored by different and, from 1a to 1c, growing percentages (63%, 87%, 99%, respectively), related to the stability of the transient carbocation, which is generated during the reaction for the different oxidized compounds.
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Scheme 1. Reaction mechanism of oxidation with RuO₄ of compounds 1a–c in α position to the nitrogen atom.

IRC analyses were performed on all the located TSs₁. In the case of the forward direction, limited to the exo attack, for 1a–c, a species very similar to an ion pair [19], with a partial character of the double bond between C-3 and N and the O-Ru-oxygen negative charged, was obtained. Additionally, in this case, for compounds 1a and 1c, the two possible diastereomeric routes were investigated. In Figure 3, the three-dimensional plots of representative ion pair structures are reported. The nature of ion pairs IP was confirmed through natural bond order (NBO) analysis (see ESI). When the transfer of the hydrogen atom to the oxygen of the ruthenium occurs, it shows a negative charge (−0.670, −0.694, −0.677 for 1a, 1b, and 1c, respectively), while the corresponding carbon is positively charged (0.205, 0.440 and 0.200 for 1a, 1b, and 1c, respectively). In all ion pairs (IP) large dipole values are detected (IP: 14.3 D; 15.5 D; 15.2 D for 1a, 1b and 1c, respectively). Starting from the ion pair, passing through a very low barrier TSI of about 2 kcal/mol (Figure 3), the products P₁ of the first step are obtained. Considering the reaction progression, the second step easily occurred with a new H transfer, with higher barriers (ΔΔG(TS₂_endo) = 12.88, 18.63, 8.26 kcal/mol, for 1a, 1b, and 1c, respectively, ΔΔG(TS₂_endo_proR) = 23.46, 22.37 kcal/mol; ΔΔG(TS₂_endo_proS) = 12.24, 11.90 kcal/mol for 1a and 1c) and so, the second step can be defined as the rate-determining one. For compound 1b, the second step of reaction takes place only in the endo position since there is no second hydrogen in the exo one. The corresponding three-dimensional plots of transition states TS₂ are reported in Figure 4.
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Starting from the ion pair, passing through a very low barrier TSI of about 2 kcal/mol (Figure 3), the products P1 of the first step are obtained. Considering the reaction progression, the second step easily occurred with a new H transfer, with higher barriers ($\Delta\Delta G_{TS2\_endo} = 12.88, 18.63, 8.26$ kcal/mol, for 1a, 1b, and 1c, respectively, $\Delta\Delta G_{TS2\_exo\_proR} = 23.46, 22.37$ kcal/mol; $\Delta\Delta G_{TS2\_exo\_proS} = 12.24, 11.90$ kcal/mol for 1a and 1c) and so, the second step can be defined as the rate-determining one. For compound 1b, the second step of reaction takes place only in the endo position since there is no second hydrogen in the exo one. The corresponding three-dimensional plots of transition states TS2 are reported in Figure 4.

Figure 2. Three-dimensional plots of the transition states TS1 for the endo and exo pathways of compounds 1a–c. Displacement vectors for TS imaginary frequencies are shown as dotted lines and distances are reported in angstroms.
Table 1. Relative free energies (kcal/mol) of TSs and percentages of the corresponding products at 298 K of the oxidation reaction with RuO$_4$ of compounds 1a–c.

| Compounds | \(\Delta G\) (kcal/mol) | % | \(\Sigma\) (endo and exo %) |
|-----------|------------------------|---|---------------------------|
| 1a        | TS1_Bn_endo            | 0.00 | 37                        | 37 |
|          | TS1_Bn_exo_proR        | 0.12 | 31                        | 63 |
|          | TS1_Bn_exo_proS        | 0.10 | 32                        |     |
| 1b        | TS1_cy_endo\(_\text{4C}_1\) | 1.13 | 8                         | 13 |
|          | TS1_cy_endo\(_\text{1C}_4\) | 1.42 | 5                         |     |
|          | TS1_cy_exo\(_\text{4C}_1\) | 0.00 | 52                        |     |
|          | TS1_cy_exo\(_\text{1C}_4\) | 0.24 | 35                        |     |
| 1c        | TS1_pOMe_endo          | 2.32 | 1                         | 1  |
|          | TS1_pOMe_exo_proR      | 0.00 | 58                        | 99 |
|          | TS1_pOMe_exo_proS      | 0.21 | 41                        |     |

When the exo attack occurs on isoxazolidines 1a–c, the possibility of an alternative route in which a second hydrogen is removed from the endo position, has been evaluated. The obtained products could be the methyl 4,5-dihydroisoxazole-5-carboxylate 4 together with benzaldehyde, cyclohexanone, and 4-metoxybenzaldehyde starting from 1a, 1b, and 1c, respectively (Scheme 2).
Figure 2. Three-dimensional plots of the transition states TS1 for the endo and exo pathways of compounds 1a–c. Displacement vectors for TS imaginary frequencies are shown as dotted lines and distances are reported in angstroms.

Figure 3. Three-dimensional plots of representative conformations of ion pairs (IP) and the corresponding transition states (TSIP) leading to P1 for compounds 1a–c. For TSs imaginary frequencies are shown as dotted lines and distances are reported in angstroms.

Figure 4. Three-dimensional plots of TS2 leading to P2 for compounds 1a–c. For TSs imaginary frequencies are shown as dotted lines and distances are reported in angstroms.

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Scheme 2. Alternative route for a second H-transfer from the endo position of isoxazolidines 1a–c.

The corresponding transition states have been located, and the 3D plots of those related to N-benzyl 1a are shown in Figure 5, as an example. However, the corresponding barriers were found to be greater than 25 kcal/mol and therefore this possibility must be excluded.
Figure 4. Three-dimensional plots of TS2 leading to P2 for compounds 1a–c. For TSs imaginary frequencies are shown as dotted lines and distances are reported in angstroms.

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Figure 5. Three-dimensional plots of TSs_sec for compounds 1a and 1c, TSs2 for compound 1b, and the corresponding products P_sec. For TSs imaginary frequencies are shown as dotted lines and distances are reported in angstroms.

Moreover, for compound 1b the second hydrogen transfer can also occur, involving one hydrogen from the β-carbon of the cyclohexyl ring, leading to H2 RuO4 and the corresponding enamine 5 which then evolves to cyclohexanone and methyl-isoxazolidine-5-carboxylate 6 (Scheme 3). However, compound 6 and cyclohexanone could be formed by hydrolysis of P1_exo_cy.

Scheme 3. Alternative route for the second H-transfer from β-position of cyclohexyl ring for the exo pathway of 1b.
We examined this route, considering the possible transfer of both equatorial and axial hydrogens, locating the corresponding transition states. Nevertheless, the calculated barriers are too high (values exceeding 30 kcal/mol) and the reaction, for the second step, cannot proceed through this pathway.

Finally, for compounds 2a–c, we also explore the possibility of a further attack of RuO$_4$ on P2-endo in position exo, considering the two different diastereomeric routes, as shown in Scheme 4. The process is not energetically demanding, with surmountable barriers that make possible the first step of the reaction ($\Delta$G(TS1 b_proR) = 13.83, 11.87 kcal/mol; $\Delta$G(TS1 b_proS) = 19.09, 16.17 kcal/mol for 2a and 2c, respectively, and $\Delta$G(TS1 b) = 16.80, 14.34 kcal/mol for 2b considering conformation $^1$C$_4$ and $^4$C$_1$, respectively), that then evolve to 7 and the corresponding carbonyl compounds.

Scheme 4. First step of the further oxidation reaction on P2-endo (2a–c), leading to 7 and corresponding carbonyl compounds.

Once obtained P1 b-exo, we also considered the possibility of the second hydrogen extraction at C-1' with the obtainment of derivatives 8 (Scheme 5). The corresponding calculated barriers are not so high ($\Delta$G(TS2 b_exo_proR) = 19.55, 16.15 kcal/mol; $\Delta$G(TS2 b_exo_proS) = 13.17, 10.80 for 2a and 2c) and the oxidation might proceed through the second step. In Figure 6, the 3D plots of the TSs1 b of the two steps and the final products P1 b obtained at the end of the reaction are reported.

Scheme 5. Oxidation reaction on P2-endo (2a,c) and P2-exo (3a,c), leading to P2 b (8).
ΔΔG(TS2 b_exo_proS ) = 13.17, 10.80 for 2a and 2c) and the oxidation might proceed through the second step. In Figure 6, the 3D plots of the TSs1 b of the two steps and the final products P1 b obtained at the end of the reaction are reported. IRC analysis in the forward direction for TS1 b_exo and TS1 b_endo showed a shoulder, corresponding to the ion pair, as determined for the main route. However, when optimized, the ion pairs fell into the energy holes corresponding to P1 b_exo and P1 b_endo.

Figure 6. Three-dimensional plots of TSs1–2b and P1 b products. TSs imaginary frequencies are shown as dotted lines and distances are reported in angstroms.
2.2. Experimental Investigation

To verify the computational outcomes, the first example we have taken into consideration was the oxidation reaction of methyl 2-benzylisoxazolidine-5-carboxylate 1a. The reaction, using 0.25 equivalent of RuO₂ and 1 equivalent of NaIO₄, in a biphasic ethyl acetate/water system, occurs in 90 min and provides a mixture of 2a, 3a, and 7 in a ratio of 22:60:18, together with a fair amount of benzaldehyde (15% yield) (Scheme 6).

![Scheme 6](image_url)

**Scheme 6.** Reagents and conditions: (i) RuO₂, NaIO₄, ethyl acetate, H₂O, rt., 90 min.

Compound 2a, according to computational data, can be easily rationalized by an endo attack of RuO₄ on the C-3 carbon of the isoxazolidine ring. On the contrary, compound 3a is formed through a C-1'(exo) attack of RuO₄ to the benzyl position of isoxazolidine 1a. Instead, the methyl 3-oxoisoxazolidine-5-carboxylate 7 derives from the further oxidation reaction of compound 2a that undergoes a debenzylation process leading also to benzaldehyde (see Schemes 1 and 4). Thus, the oxidation reaction of 1a proceeds with an exo/endo selectivity of 3:2, showing that this selectivity, according to in silico studies, is controlled by carbocation stability (benzyl vs. secondary carbocation).

Then, following the computational results reported in Table 1, we explored the oxidation reaction of methyl 2-cyclohexyl isoxazolidine-5-carboxylate 1b, where it is expected that the formation of a tertiary carbocation intermediate is able to increase the exo selectivity. The reaction, performed in the same conditions reported in Scheme 6, gave a mixture of compounds 2b, 6, and 7 in a 14:80:6 ratio together with cyclohexanone (Scheme 7). As expected, isoxazolidin-3-one 2b is produced by an endo attack of RuO₄ on 1b following the route reported in Scheme 1. Compound 6 is probably formed by hydrolysis of P1_exo_cy (Scheme 3) after exo oxidation of compound 1b, while compound 7 is produced from 2b by elimination of cyclohexyl group (Scheme 4). Additionally, for this reaction, the experimental results agree with computational outcomes affording a 4:1 exo/endo selectivity (tertiary vs. secondary carbocation).

![Scheme 7](image_url)

**Scheme 7.** Reagents and conditions: (i) RuO₂, NaIO₄, ethyl acetate, H₂O, rt., 90 min.

The regioselectivity of RuO₄ oxidation favoring the exo attack was also proven on methyl 2-(4-methoxybenzyl) isoxazolidine-5-carboxylate 1c, which contains an electron donor group in the para position of benzyl group (Scheme 8). The reaction, performed following the same synthetic protocol, afforded compound 3c in 74.8% yield, 4-methoxybenzaldehyde, and a low amount of 2c and 7 (5.95% and 4.25 % yield, respectively). The formation of 2c, 3c, and 7 are amenable following the routes described in Schemes 1 and 4. For this reaction, the exo/endo ratio was found to be about 9:1, in good agreement with the computational data (4-methoxybenzyl vs. secondary carbocation) (See Supplementary Materials).
Then, following the computational results reported in Table 1, we obtained 1a-c (total yield 85%), from 1b we obtained 2b, 6, and 7 (total yield 80%), from 1c we obtained 2c, 3c, and 7 (total yield 85%).

Methyl 2-benzyl-3-oxoisoxazolidine-5-carboxylate (2a): pale yellow oil, yield 18.70%. 

3. Materials and Methods

3.1. Computational Methods

All calculations were performed using the Gaussian16 program package [20]. Optimizations were performed using the B3 LYP functional [21,22] in conjunction with Grimme’s dispersion correction [23,24] (henceforth referred to as B3 LYP-d3) chosen referring to similar systems’ studies [18,19]. The standard basis set Def2 SVP was employed [25,26]. Solvent effects on water, using the C-PCM method [27,28] were taken into consideration. The reaction pathways were confirmed by IRC analyses performed at the same level as above. Vibrational frequencies were computed at the same level of theory to define the optimized structures as minima or transition states, which present an imaginary frequency corresponding to the forming bonds. Thermodynamics at 298.15 K allowed Gibb’s free energies to be calculated.

3.2. General

Solvents and reagents were used as received from commercial sources. NMR spectra (1H-NMR recorded at 500 MHz, 13C-NMR recorded at 125 MHz) were obtained in CDCl₃ solution on a Varian instrument (Agilent Technologies, Palo Alto, CA, USA), and data are reported in ppm relative to TMS as an internal standard. Elemental analyses were performed with a Perkin Elmer elemental analyzer (PerkinElmer, Waltham, MA, USA). MW-assisted reactions were performed on a CEM Discover instrument equipped with electromagnetic stirring and an IR probe used for external temperature control (CEM Corporation, NC, USA). Thin-layer chromatographic separations were carried out on Merck silica gel 60-F254 precoated aluminum plates (Merck, Darmstadt, Germany). Preparative separations were carried out using a Büchi C-601 MPLC instrument (BUCHI Italia S.r.l., Milano, Italy) using Merck silica gel 0.040–0.063 mm, and the eluting solvents were delivered by a pump at the flow rate of 3.5–7.0 mL/min. All solvents were dried according to methods in the literature. Isoxazolidines 1a-c have been synthesized according to standard procedures [8,35].

3.3. General Procedure for RuO₂/NaIO₄ Oxidation

To a solution of NaIO₄ (1 mmol) in water (30 mL) was added RuO₂ (0.25 mmol) under nitrogen. The resulting green–yellow solution was stirred for 30 min and was followed by addition of isoxazolidine 1a-c (0.90 mmol) in EtOAc (30 mL) in one portion. The solution remained yellowish during the reaction. After 90 min of stirring at room temperature, the mixture was diluted with EtOAc and filtered through a pad of Celite. The organic layer was washed with saturated NaHSO₄, which resulted in precipitation of black Ru. The precipitate was filtered off through a pad of Celite. The EtOAc layer was washed with brine and dried with anhydrous Na₂SO₄; the solvent was removed by evaporation in a rotary evaporator to obtain the crude product. All products were purified by MPLC chromatography. From 1a we obtained 2a, 3a, and 7 (total yield 85 %), from 1b we obtained 2b, 6, and 7 (total yield 80 %), from 1c we obtained 2c, 3c, and 7 (total yield 85%).
Methyl 2-cyclohexyl-3-oxoisoxazolidine-5-carboxylate (2b): yellow oil, 11.20% yield. $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 4.83 (t, J = 4.7 Hz, 1 H), 3.82–3.66 (m, 4 H), 3.16–2.95 (m, 2 H), 1.92–1.75 (m, 4 H), 1.66–1.36 (m, 6 H) ppm. $^{13}$C NMR (126 MHz, CDCl$_3$) $\delta$ 171.45, 171.11, 73.18, 57.02, 52.37, 36.60, 29.93, 26.25, 24.42. Anal. Calcd for C$_{13}$H$_{17}$NO$_3$: C, 58.14; H, 7.54; N, 6.16; found C, 58.15; H, 7.51; N, 6.12.

Methyl 2-(4-methoxybenzyl)-3-oxoisoxazolidine-5-carboxylate (2c): pale yellow oil, 5.95% yield. $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 7.18 (d, J = 6.8 Hz, 2 H), 6.78 (d, J = 8.6 Hz, 2 H), 4.78 (dd, J = 9.6, 5.2 Hz, 1 H), 4.64 (dd, J = 15.5 Hz, 1 H), 4.52 (d, J = 15.5 Hz, 1 H), 3.70 (s, 3 H), 3.65 (s, 3 H), 3.03 (dd, J = 16.8, 5.2 Hz, 1 H), 2.90 (dd, J = 16.8, 5.2 Hz, 1 H) ppm. $^{13}$C NMR (126 MHz, CDCl$_3$) $\delta$ 169.83, 169.58, 159.40, 129.68, 126.85, 113.96, 74.22, 55.28, 52.80, 44.36, 35.91 ppm. Anal. Calcd for C$_{13}$H$_{15}$NO$_5$: C, 58.86; H, 5.70; N, 5.28; found C, 58.83; H, 5.68; N, 5.25.

Methyl 2-benzoylisoxazolidine-5-carboxylate (3a): white sticky oil, 51%. $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 7.85–7.80 (m, 2 H), 7.50–7.44 (m, 1 H), 7.43–7.38 (m, 2 H), 4.68–4.64 (m, 1 H), 4.12–4.03 (m, 1 H), 3.86–3.82 (m, 1 H), 3.64 (s, 3 H), 2.66–2.50 (m, 2 H) ppm. $^{13}$C NMR (126 MHz, CDCl$_3$) $\delta$ 170.31, 167.06, 145.39, 133.44, 128.10, 127.94, 77.25, 52.87, 49.09, 31.26 ppm. Anal. Calcd for C$_{13}$H$_{15}$NO$_4$: C, 61.27; H, 5.57; N, 5.95; found C, 61.28; H, 5.56; N, 5.94.

Methyl 2-(4-methoxybenzyl)isoxazolidine-5-carboxylate (3b): white sticky oil, 48.8% yield. $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 7.78 (d, J = 8.9 Hz, 2 H), 6.82 (d, J = 8.9 Hz, 2 H), 4.58 (dd, J = 8.5, 4.1 Hz, 1 H), 3.95 (dd, J = 10.8, 8.7, 7.1 Hz, 1 H), 3.75 (s, 3 H), 3.79–3.71 (m, 1 H), 3.56 (s, 3 H), 2.57–2.39 (m, 2 H) ppm. $^{13}$C NMR (126 MHz, CDCl$_3$) $\delta$ 170.78, 170.27, 162.07, 131.34, 125.38, 113.10, 77.14, 55.29, 52.36, 48.41, 31.09 ppm. Anal. Calcd for C$_{13}$H$_{15}$NO$_4$: C, 58.86; H, 5.70; N, 5.28; found C, 58.84; H, 5.68; N, 5.29.

Methyl isoxazolidine-5-carboxylate (6): white sticky oil, yield 64%. $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 4.96 (bs, 1 H), 4.59 (t, J = 3.9 Hz, 1 H), 3.73 (s, 3 H), 3.38–3.26 (m, 1 H), 3.27–3.13 (m, 1 H), 2.23–2.08 (m, 2 H) ppm. $^{13}$C NMR (126 MHz, CDCl$_3$) $\delta$ 172.25, 77.32, 52.34, 47.82, 29.52 ppm. Anal. Calcd for C$_5$H$_9$NO$_3$: C, 45.80; H, 6.92; N, 10.68; found C, 45.77; H, 6.91; N, 10.62.

Methyl 3-oxoisoxazolidine-5-carboxylate (7): white sticky oil (from 1 a, 15.30% yield; from 1 b, 4.8% yield; from 1 c, 4.25% yield). $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 8.25 (bs, 1 H), 4.46 (dd, J = 6.1, 5.0 Hz, 1 H), 3.85 (s, 3 H), 2.87 (dd, J = 16.8, 5.0 Hz, 1 H), 2.79 (dd, J = 16.8, 6.1 Hz, 1 H) ppm. $^{13}$C NMR (126 MHz, CDCl$_3$) $\delta$ 171.91, 170.32, 66.65, 53.27, 23.39 ppm. Anal. Calcd for C$_5$H$_7$NO$_4$: C, 41.38; H, 4.86; N, 9.65; found C, 41.35; H, 4.85; N, 9.66.

4. Conclusions

In this paper, we performed a computational mechanistic study of Ru$_4$-catalyzed oxidation of differently N-substituted isoxazolines 1a–c. Based on our previous results, we first considered the C-3(endo)/C-1'(exo) selectivity, following the supposed [3 + 2] one-step, but asynchronous reaction mechanism with a double hydrogen transfer. In analogy with previously reported results [19], the energy barrier of the second transfer, for compounds 1a and 1c, is higher than that of the first one and so the second transfer can be defined as rate-determining. On the other hand, the first one rules the regioselectivity of the reaction and is considered the regioselectivity-determining step.

From the first hydrogen transfer, passing a low energy barrier, P$_1$-endo and P$_1$-exo are obtained. These intermediates evolve to 2a and 3a, after a second hydrogen extraction, resulting in the final compounds oxidized in C-3 or C-1', respectively. For all compounds 1a–c the exo attack is preferred to the endo one, showing for the corresponding oxidated product percentages growing from 1a to 1c. The computationally determined selectivity in parallel reflects the stability order of transient carbocations involved in the ion pairs. These carbocations were found along the reaction pathway and confirmed by NBO analysis. Once obtained, P$_1$-exo can undergo hydrolysis, affording methyl 4,5-dihydroisoxazole-5-carboxylate 4 together with benzaldehyde, cyclohexanone, or 4-methoxybenzaldehyde.
Based on theoretical results, products 2a, c, and 3a, c can still react with RuO₄, giving a new [3 + 2] one-step process with the obtaining of the dicarbonyl derivative 8. Nevertheless, the product of the first step P₁ b can be hydrolyzed before the second oxidation, generating the methyl 3-isoxazolidinone-5-carboxylate 7.

For the second reaction step, the possibility of hydrogen extraction in alternative positions was examined, but the energy barrier results were too high.

Finally, the computational outcomes have been experimentally confirmed. For all the investigated reactions, the exo attack is preferred to the endo one, confirming that the oxidation selectivity is strictly related to the stability order of transient carbocations found along the reaction pathway.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/molecules27175390/s1, Figure S1: ¹H NMR of 2a; Figure S2: ¹³C NMR of 2a; Figure S3: ¹H NMR of 2b; Figure S4: ¹³C NMR of 2b; Figure S5: ¹H NMR of 2c; Figure S6: ¹³C NMR of 2c; Figure S7: ¹H NMR of 3a; Figure S8: ¹³C NMR of 3a; Figure S9: ¹H NMR of 3c; Figure S10: ¹³C NMR of 3c; Figure S11: ¹H NMR of 6; Figure S12: ¹³C NMR of 6; Figure S13: ¹H NMR of 7; Figure S14: ¹³C NMR of 7; Figure S15: NBO analysis of ion pair IP located along the reaction pathway of compounds 1a–c; B3LYP/def2svp/emp=gd3bj/int=ultrafine/solvent=water cartesian coordinates; Table S1: Free energies and imaginary frequencies for transition states to the oxidation reaction of 1a; Table S2: Free energies and imaginary frequencies for transition states to the oxidation reaction of 1b; Table S3: Free energies and imaginary frequencies for transition states to the oxidation reaction of 1b.

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