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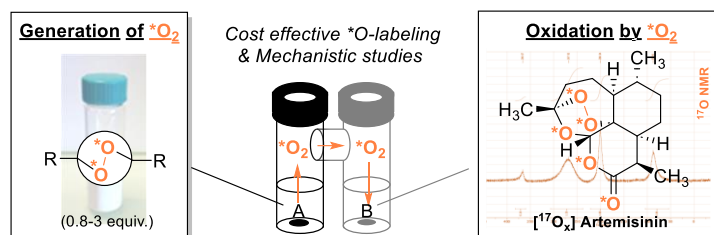
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# *Ex Situ* Generation of $^{18}\text{O}_2$ and $^{17}\text{O}_2$ from Endoperoxides for $^*\text{O}$ -Labeling and Mechanistic Studies of Oxidations by Dioxygen

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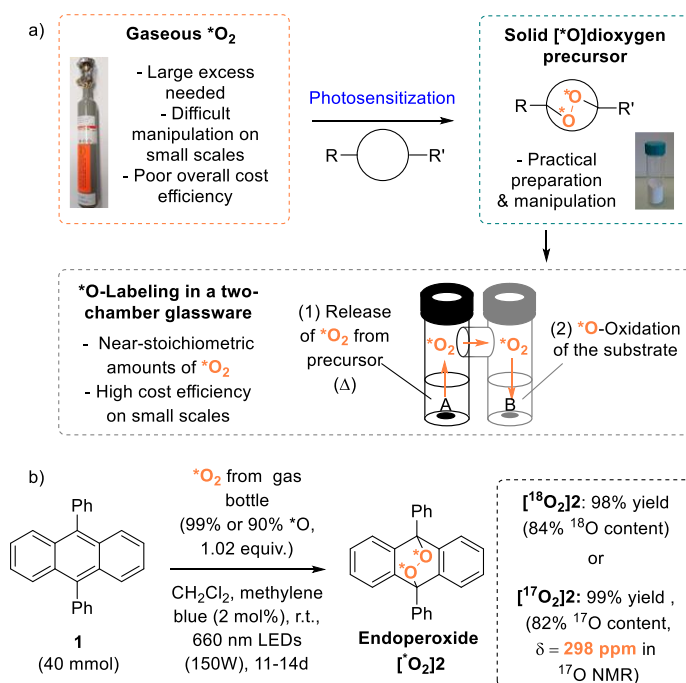
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**ABSTRACT:** Near-stoichiometric amounts of  $^{18}\text{O}_2$  &  $^{17}\text{O}_2$  were generated *ex situ* from endoperoxides in a two-chamber glassware to oxidize various substrates. This strategy gave  $^*\text{O}_2$  endoperoxides,  $^*\text{O}_1$  quinones,  $^*\text{O}_1$  phenols and  $^*\text{O}_x$  artemisinin in moderate to good yields and high isotopic enrichments (up to 83%) at affordable costs. Moreover, mass spectrometry and  $^{17}\text{O}$  NMR of the  $^*\text{O}$  products provided valuable information on the chemical mechanisms involved

Among the various elements, oxygen plays a key role in many functional groups, and its isotopic labeling often proves determinant for mechanistic insights.<sup>1</sup> Indeed,  $^{18}\text{O}$  can be easily differentiated by mass analysis from the predominant  $^{16}\text{O}$  (99.759%), and recent advances in NMR instrumentation allows efficient detection of the chemical shifts of  $^{17}\text{O}$  (-30 to +1000 ppm).<sup>2</sup> Due to the extremely low natural abundance of  $^{18}\text{O}$  and  $^{17}\text{O}$  (0.204% and 0.037%, respectively), the use of isotopically enriched compounds is essential, and synthetic methodologies for the incorporation of labeled oxygen ( $^*\text{O}$ ) have been extensively studied.<sup>3,4,5</sup> They generally rely on one of the cheapest isotope precursors:  $^*\text{O}]\text{H}_2\text{O}$ , but often require harsh conditions limiting their use to simple synthons, and/or involve reversible isotopic exchange yielding moderate isotopic enrichments. Advanced isotopic oxygen sources ( $^*\text{O}]\text{CO}_2$ ,  $^*\text{O}]\text{CH}_3\text{OH}$ ,...) are compatible with more specific reactions, but are extremely costly due to their low availability. Interestingly, some examples also reported the use of gaseous  $^*\text{O}_2$ ,<sup>3,6</sup> for which the molar cost of the  $^*\text{O}$ -labeled atoms is comparable to  $^*\text{O}]\text{H}_2\text{O}$ .<sup>7</sup> However, the need to employ large excesses of this gas and the difficulty to manipulate it precisely greatly increase the overall cost of these procedures, which made them under-used. All isotopically labeled gases share these drawbacks, but as demonstrated in the literature for some examples such as  $^*\text{C}]\text{CO}$ ,<sup>8</sup>  $^*\text{H}_2$ ,<sup>9</sup> or  $^*\text{C}_2^*\text{H}_4$  ethylene,<sup>10</sup> they might be solved using a two-chamber glassware and a stable precursor able to release “*ex situ*” stoichiometric amounts of the desired gas. Thus, we decided to investigate the *ex situ* generation of  $^*\text{O}_2$  from easy-to-access solid  $^*\text{O}]\text{compounds}$  to enable cost-efficient  $^*\text{O}$ -labeling (Scheme 1, a).

**Scheme 1: Strategy envisaged for efficient  $^*\text{O}$ -labeling with  $^*\text{O}_2$  and syntheses of EPO  $^{18}\text{O}_2\text{]2}$  and  $^{17}\text{O}_2\text{]2}$ .**

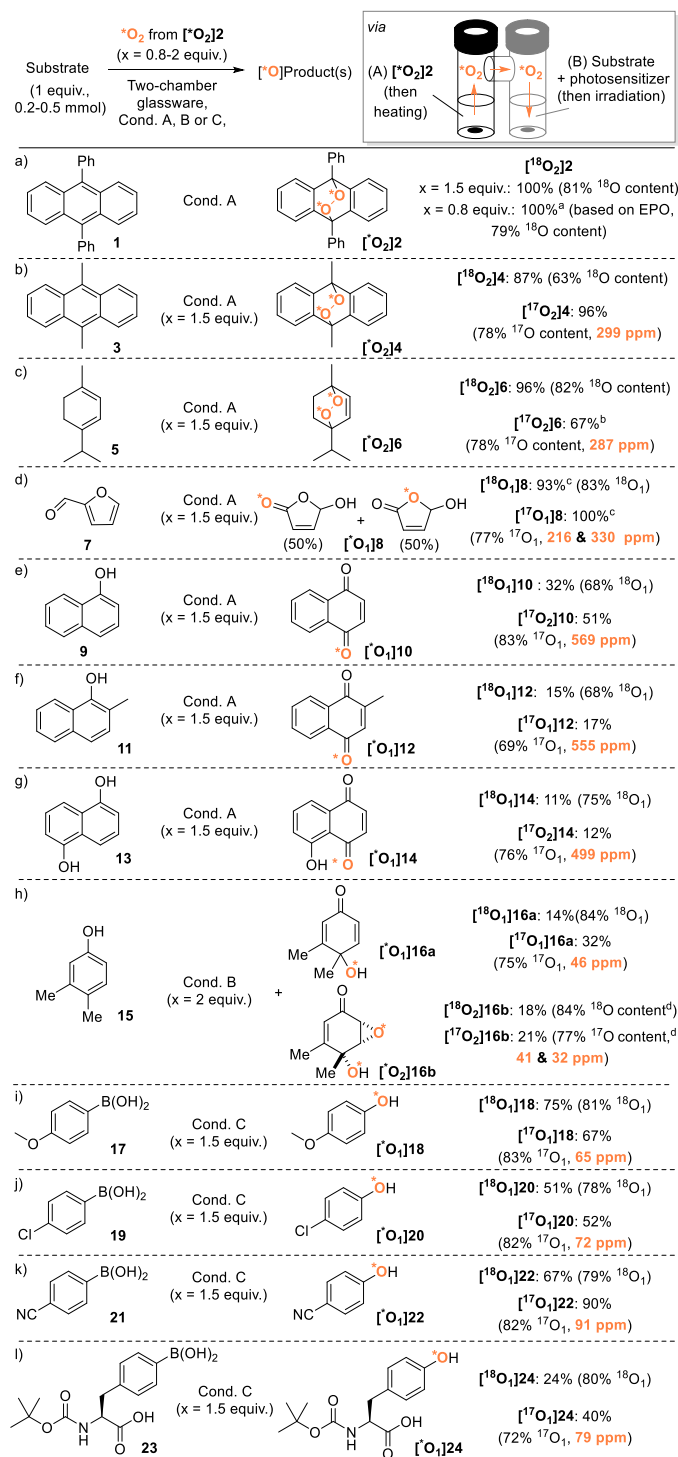


Notably, endoperoxides scaffolds (EPO) are known to be precursors of singlet oxygen,<sup>11,12,13</sup> and among the various possibilities tested, endoperoxide **2** was selected as the most promising structure. Indeed, it possessed both the ability to generate dioxxygen quantitatively when heated over 100°C,<sup>14</sup> and a high stability at room temperature, which avoided specific storage conditions and ensured practical manipulations. Moreover, multi-gram quantities of its  $^*\text{O}$ -labeled versions  $^{18}\text{O}_2\text{]2}$  and  $^{17}\text{O}_2\text{]2}$  were obtained in almost quantitative yields by reacting the commercially available 9,10-diphenylanthracene **1** with photosensitized singlet  $^{18}\text{O}_2$  coming from a low-pressure gas bottle (1L, 1.02 equiv., Scheme 1, b). Interestingly, the isotopic loss in this step was moderate, and starting from 90-99%  $^*\text{O}$ -labeled dioxxygen provided the desired EPOs with high isotopic enrichments of 84% and 82% for  $^{18}\text{O}_2\text{]2}$  and  $^{17}\text{O}_2\text{]2}$ , respectively.<sup>15</sup> Then, the use of these compounds for  $^*\text{O}$ -labeling on small scales (0.2-0.5 mmol) was explored in combination with a specific two-chamber glassware designed to allow independent heating/light irradiation of each chamber.<sup>16</sup> Firstly, the efficiency of the  $^*\text{O}_2$  generation & transfer was investigated by adding 1.5 equiv. of  $^{18}\text{O}_2\text{]2}$  in chamber A and 9,10-diphenylanthracene **1** (1 equiv.) in chamber B in dichloromethane in presence of a catalytic amount of methylene blue (Scheme 2, a). After flushing the system under  $\text{N}_2$  by freeze-and-pump cycles, chamber A was heated over 150°C for 3 min.<sup>17</sup> Then, chamber B was irradiated at r.t. for 66 h hours under red light (660 nm LEDs), and  $^{18}\text{O}_2\text{]2}$  could be isolated from this chamber in quantitative yield after a simple filtration on an alumina pad. Moreover, the excellent isotopic enrichment of 81% determined for  $^{18}\text{O}_2\text{]2}$  indicated minimal isotopic losses (Scheme 2, a).<sup>18</sup> Switching to a limiting amount of starting  $^{18}\text{O}_2\text{]2}$  in chamber A also gave in chamber B  $^{18}\text{O}_2\text{]2}$  in quantitative yield (based on the initial EPO) with 79%  $^*\text{O}$ -labeling, which demonstrated the excellent efficiency of this set-up. Then, the scope of these oxidations by  $^*\text{O}$ -labeled singlet dioxxygen was explored, and various substrates were engaged with only a slight excess of  $^{18}\text{O}_2\text{]2}$  or  $^{17}\text{O}_2\text{]2}$  (1.5 to 2 equiv.). When 9,10-dimethylantracene **3** was employed as starting material in conditions A at -10°C, the desired  $^{18}\text{O}_2\text{]4}$  and  $^{17}\text{O}_2\text{]4}$  were also obtained in very good yields of 87% and 96%, respectively (Scheme 2, b). These results demonstrated the reproducibility of the procedure, despite observing slightly lowered  $^*\text{O}$ -contents in this case (63%-78%). Then, the synthesis  $^{18}\text{O}_2\text{]6}$  &  $^{17}\text{O}_2\text{]6}$  were produced easily in 96% and 67% yields with excellent isotopic contents of  $^{18}\text{O}/^{17}\text{O}$  ranging from 78% to 82% (Scheme 2, c). The oxidation of furfural **7** was also attempted using 1.5 equiv. of  $^{18}\text{O}_2\text{]2}$  or  $^{17}\text{O}_2\text{]2}$ , and 5-hydroxy-2(5H)-furanone<sup>20</sup> was obtained with excellent yields (93% and 100%, Scheme 2, d). Mass analyses determined that the main isotopic molecular formula was  $\text{C}_4\text{H}_4\text{O}_2^*\text{O}_1$  for both (83% & 77%), which indicated minimal

isotopic losses. However,  $^{17}\text{O}$  NMR spectrum of [ $^{17}\text{O}_1$ ]**8**, revealed the presence of two signals with identical integrations. As previously described in the literature,<sup>21</sup> this confirmed that either the carbonyl- or the intracyclic- $^*\text{O}$ -atom was labeled, the two isotopomers being in equilibrium *via* the transient opening of the lactone.<sup>16</sup> Oxidation of naphthoquinol **9** was also explored, and the mono- $^*\text{O}$ -labeled naphthoquinones [ $^{18}\text{O}_1$ ]**10** and [ $^{17}\text{O}_1$ ]**10** were synthesized in one step using conditions A. Yields were moderate (32-51%), but high isotopic enrichments were again measured (68%-83%, Scheme 2, e). Applying directly these conditions to substituted naphthoquinols **11** and **13** also gave the desired quinones, but yields were only ranging from 11% to 17%. However, this method still provided a straightforward access to the isotopically labeled versions of menadione (vitamin K3)<sup>22</sup> [ $^*\text{O}_1$ ]**12** and juglone<sup>23</sup> [ $^*\text{O}_1$ ]**14** with high  $^*\text{O}$ -enrichments (68-76%, Scheme 2, f & g). Moreover,  $^{13}\text{C}$  NMR demonstrated that the desired carbonyl possessed the sole  $^{18}\text{O}$ -labeled atom in [ $^{18}\text{O}_1$ ]**12** and [ $^{18}\text{O}_1$ ]**14**.<sup>16</sup>

The photooxidation of substituted phenols was also investigated by irradiating substrate **15** under green light in presence of catalytic amounts of rose bengal & cesium carbonate,<sup>24</sup> while being exposed to  $^*\text{O}$ -labeled dioxygen generated from two equivalents of [ $^*\text{O}_2$ ]**2**. A mixture of quinol [ $^*\text{O}_1$ ]**16a** and epoxyquinol [ $^*\text{O}_2$ ]**16b** was obtained in both cases, with overall yields of 32% to 53% (Scheme 2, h). Isotopic enrichments of 84% and 77%, were determined for [ $^{18}\text{O}_1$ ]**16a** and [ $^{17}\text{O}_1$ ]**16a**, respectively. These values indicated very limited isotopic losses from the parent EPO [ $^*\text{O}_2$ ]**2**, and  $^{17}\text{O}$  NMR of [ $^{17}\text{O}_1$ ]**16a** confirmed the unique labeling at the hydroxyl group. On the other hand, despite an overall  $^{18}\text{O}$ -content of 77% for the alcohol and epoxide functions of [ $^{18}\text{O}_x$ ]**16b**, the distribution of the isotopic molecular formulas was determined as 72% of  $\text{C}_8\text{H}_{10}^{16}\text{O}_1^{18}\text{O}_2$ , 24% of  $\text{C}_8\text{H}_{10}^{16}\text{O}_2^{18}\text{O}_1$  and 4% of  $\text{C}_8\text{H}_{10}^{16}\text{O}_3$ .<sup>16</sup> Taking in account the isotopic distribution of the initial endoperoxide of 84% of [ $^{18}\text{O}_2$ ]**2** and 16% of [ $^{16}\text{O}_2$ ]**2**,<sup>16</sup> the high ratio of mono- $^*\text{O}$ -labeled compound [ $^{18}\text{O}_1$ ]**16b** observed was more consistent with an independent labeling of the two oxygen atoms rather than with an intramolecular double-oxidation pathway. Similar ratios were also measured for [ $^{17}\text{O}_x$ ]**16b**, and the  $^{17}\text{O}$  NMR experiment revealed that the two signals of the hydroxy and epoxy groups had equivalent integrations.<sup>16</sup> Taken together, these results demonstrated that the epoxidation of the double bond mainly occurred by an intermolecular reaction with the hydroperoxide intermediate generated after the first step of phenol oxidation.<sup>24c</sup> Finally, the photooxidation of boronic acids into phenols<sup>25</sup> was investigated using this two-chamber set-up with 1.5 equivalent of  $^*\text{O}$ -labeled endoperoxide [ $^*\text{O}_2$ ]**2**. Interestingly, irradiation of arylboronic acids **17**, **19** and **21** under green light in presence of 40 mol% of triethylamine and 5 mol% of rose bengal in ethanol at r.t. for 36 h provided smoothly the desired  $^*\text{O}$ -phenols [ $^*\text{O}_1$ ]**18**, [ $^*\text{O}_1$ ]**20** and [ $^*\text{O}_1$ ]**22**. Especially, good yields ranging from 51% to 90% and excellent isotopic enrichments of 78-83% were obtained (Scheme 2, i, j & k). These results confirmed that the labeled  $^*\text{O}$ -atom introduced originated from of  $\text{O}_2$  even at a low partial pressure. Moreover, these conditions were also applied to prepare in one-step [ $\eta$ - $^{17}\text{O}_1$ ]-(*S*)-*N*-Boc-tyrosine **24** in 40% yield and 72% of  $^{17}\text{O}$ -labeling at the phenol position (Scheme 2, l), which, after *N*-Boc-deprotection, can be used to study protein structures by  $^{17}\text{O}$  NMR.<sup>26</sup>

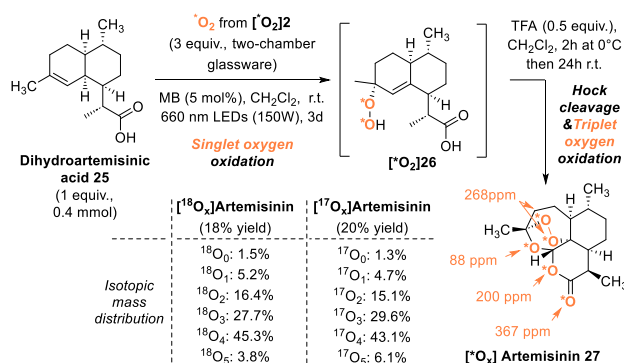
**Scheme 2: Oxidations of various substrates by near-stoichiometric amounts of  $^*\text{O}_2$  produced *ex situ* from EPO [ $^{18}\text{O}_2$ ]2 or [ $^{17}\text{O}_2$ ]2.**



*General protocol:* [ $^*\text{O}_2$ ]2 (x equiv.) was placed in chamber A, substrate (0.2 to 0.4 mmol) & photosensitizer were solubilized in the solvent in chamber B. The system was flushed by  $\text{N}_2$  using freeze-and-pump cycles. Chamber A was heated over  $150^\circ\text{C}$  for 3 min, then chamber B was irradiated by a suitable light. Conditions A: methylene blue (5 mol%),  $\text{CH}_2\text{Cl}_2$  or  $\text{CH}_3\text{OH}$ , r.t. or  $-10^\circ\text{C}$ , 660 nm LEDs (150W), 2 d. Conditions B:  $\text{Cs}_2\text{CO}_3$  (15 mol%), rose bengal (1 mol%), MeOH, r.t., 525 nm LEDs (40W), 36-48h. Conditions C:  $\text{NEt}_3$  (40 mol%), rose bengal (5 mol%), EtOH, r.t., 525 nm LEDs (40W), 36h. Isotopic enrichments determined by mass analyses.  $\delta(^{17}\text{O})$  in orange. See SI for detailed conditions. <sup>a</sup>Isolated in mixture with **1**. <sup>b</sup>Isolated in mixture with 16% of *p*-cymene. <sup>c</sup>5-hydroxy-2(5H)-furanone [ $^*\text{O}$ ]8 converted partially converted into [ $^*\text{O}$ ]-*trans*- $\beta$ -formylacrylic acid after isolation <sup>d</sup>Calculated on the  $^*\text{O}$ -atoms introduced.

Finally, the possibility to perform complex and/or multiple oxidations was investigated, and artemisinin **27** was identified as a suitable molecular target. Indeed, this well-studied anti-malarial drug is a natural endoperoxide compound<sup>27</sup> that can be obtained from dihydroartemisininic acid **25** by two consecutive dioxygen oxidations involving a postulated intermediary Hock cleavage.<sup>28</sup> In this context, compound **25** was placed in CH<sub>2</sub>Cl<sub>2</sub> under N<sub>2</sub> in the chamber B of the two-chamber glassware with 5 mol% of methylene blue, while chamber A was previously loaded with three equivalents of [<sup>\*</sup>O<sub>2</sub>]**2** (Scheme 3). After thermal release of <sup>18</sup>O<sub>2</sub> or <sup>17</sup>O<sub>2</sub>, chamber B was irradiated under red light at r.t. for three days to enable the desired ene-reaction that formed *in situ* endoperoxide [<sup>\*</sup>O<sub>2</sub>]**26**. In a second step, trifluoroacetic acid was added to the reaction mixture via a gas tight syringe to favor the one-pot Hock cleavage leading to the enol. Interestingly, it could react directly with the residual <sup>\*</sup>O-labeled triplet oxygen in the two-chamber glassware, and spontaneous cyclization occurred to give the desired <sup>\*</sup>O-labeled versions of artemisinin [<sup>18</sup>O<sub>x</sub>]**27** and [<sup>17</sup>O<sub>x</sub>]**27** in decent isolated yields of 18% and 20%, respectively.

**Scheme 3: Synthesis of [<sup>\*</sup>O<sub>x</sub>]Artemisinin **27** by one-pot double oxidation of dihydroartemisininic acid **25** using <sup>\*</sup>O<sub>2</sub> produced *ex situ* from 3 equivalents of [<sup>18</sup>O<sub>2</sub>]**2** or [<sup>17</sup>O<sub>2</sub>]**2**.**

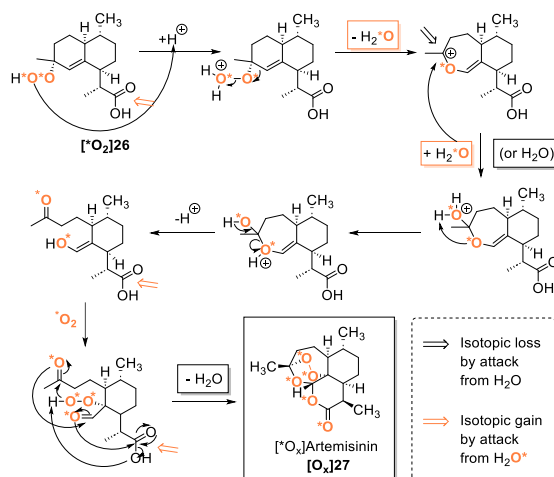


MB: methylene blue. Isotopic distribution determined by mass analyses.  $\delta(^{17}\text{O})$  in orange. See SI for exact experimental conditions.

In both cases, mass analyses revealed a mixture of isotopic formulas ranging from zero to five oxygen atoms labeled, yet the expected [<sup>\*</sup>O<sub>4</sub>]**27** was the main specie detected in 43% to 45%. Detailed examination of the isotopic mass distribution for the <sup>18</sup>O-labeled artemisinin showed that summing the proportions for [<sup>18</sup>O<sub>4</sub>]**27** & [<sup>18</sup>O<sub>3</sub>]**27** (= 73%) and [<sup>18</sup>O<sub>2</sub>]**27** & [<sup>18</sup>O<sub>1</sub>]**27** (= 22%) gave values close to the expected results (i.e. 71% of [<sup>18</sup>O<sub>4</sub>]**27**, 26% of [<sup>18</sup>O<sub>2</sub>]**27** and 3% of [<sup>18</sup>O<sub>0</sub>]**27**). This suggested that one <sup>\*</sup>O-labeled atom was lost partially during the double oxidation process. These results confirmed the Hock mechanism (Scheme 4), which involved the <sup>\*</sup>O-<sup>\*</sup>O bond cleavage of [<sup>\*</sup>O<sub>2</sub>]**26** *via* protonation of the terminal oxygen of the peroxide and consecutive [<sup>\*</sup>O]H<sub>2</sub>O departure. Indeed, the water molecule is recaptured in these case by the carbocation issued from the vinyl migration. Thus, as unlabeled H<sub>2</sub>O is in the final cyclization cascade, competition occurred at this stage between [<sup>\*</sup>O]H<sub>2</sub>O and [<sup>16</sup>O]H<sub>2</sub>O to trap the carbocation, which decreased partially from one the number of <sup>\*</sup>O-labeled atoms in the final [<sup>\*</sup>O<sub>x</sub>]artemisinin **27**. Moreover, the unreacted <sup>\*</sup>O-labeled water generated was able to freely exchange with the <sup>\*</sup>O-atoms of the carboxylic acid until the cyclization step, which explains the small amounts of [<sup>18</sup>O<sub>5</sub>]**27** detected (3.8%, Scheme 3). Finally, the <sup>17</sup>O NMR spectra of [<sup>17</sup>O<sub>x</sub>]artemisinin **27** showed the presence of three main signals (endoperoxide at 268 ppm, O-ester at 200 ppm and O-acetal at 88 ppm) and a small signal at 367 ppm, confirming a minimal amount of <sup>\*</sup>O-atoms in the C=O bond.<sup>16</sup>

To conclude, easy-to-handle EPOs [<sup>18</sup>O<sub>2</sub>]**2** and [<sup>17</sup>O<sub>2</sub>]**2** were synthesized and used to produce near-stoichiometric amounts of <sup>\*</sup>O<sub>2</sub> *ex situ* in a two-chamber glassware. This strategy allowed the <sup>18</sup>O- and <sup>17</sup>O-labeling of various compounds on small scales with very high isotopic enrichments and at affordable costs. Particularly, analyzing the [<sup>\*</sup>O]products by mass spectrometry and <sup>17</sup>O NMR provided valuable information on the chemical mechanisms involved. Thus, we believe that this method will bring significant benefits for studies on oxidations with O<sub>2</sub>.

#### Scheme 4: Proposed mechanism for the formation of [<sup>x</sup>O<sub>x</sub>]artemisinin from peroxide [<sup>x</sup>O<sub>2</sub>]26



#### ASSOCIATED CONTENT

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##### Supporting Information

General experimental procedures and analysis/spectroscopic data for new compounds (<sup>1</sup>H NMR, <sup>13</sup>C NMR, <sup>17</sup>O NMR and mass spectra). The Supporting Information is available free of charge on the ACS Publications website as PDF file ([https://pubs.acs.org/doi/suppl/10.1021/acs.orglett.3c01487/suppl\\_file/o13c01487\\_si\\_001.pdf](https://pubs.acs.org/doi/suppl/10.1021/acs.orglett.3c01487/suppl_file/o13c01487_si_001.pdf)).

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##### Author Contributions

P. H. conceptualized the project and acquired the funding. Investigation and methodology were performed by A. D. & M.-F. B., and P. H. & E. F. supervised the study. P. H. wrote the original draft and A. D., M.-F. B. and E. F. reviewed and edited the draft.

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