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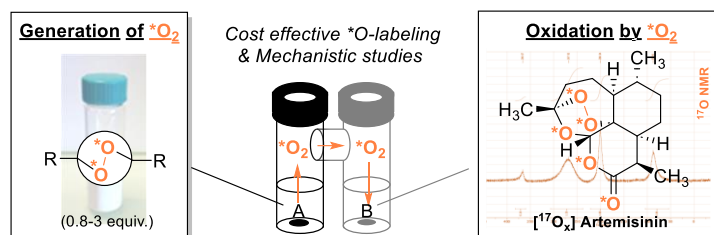
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Ex Situ Generation of $^{18}\text{O}_2$ and $^{17}\text{O}_2$ from Endoperoxides for $^*\text{O}$ -Labeling and Mechanistic Studies of Oxidations by Dioxygen

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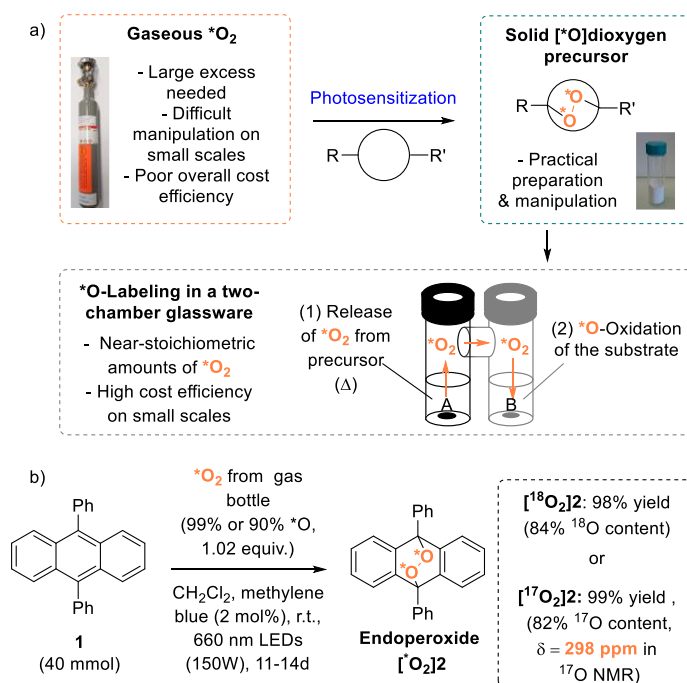
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ABSTRACT: Near-stoichiometric amounts of $^{18}\text{O}_2$ & $^{17}\text{O}_2$ were generated *ex situ* from endoperoxides in a two-chamber glassware to oxidize various substrates. This strategy gave $^*\text{O}_2$ endoperoxides, $^*\text{O}_1$ quinones, $^*\text{O}_1$ 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}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.¹ Indeed, ^{18}O can be easily differentiated by mass analysis from the predominant ^{16}O (99.759%), and recent advances in NMR instrumentation allows efficient detection of the chemical shifts of ^{17}O (-30 to +1000 ppm).² Due to the extremely low natural abundance of ^{18}O and ^{17}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.^{3,4,5} 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 $^*\text{O}_2$,^{3,6} for which the molar cost of the $^*\text{O}$ -labeled atoms is comparable to $^*\text{O}(\text{H}_2\text{O})$.⁷ 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 $^*\text{C}(\text{CO})$,⁸ $^*\text{H}_2$,⁹ or $^*\text{C}_2^*\text{H}_4$ ethylene,¹⁰ 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 $^*\text{O}$ 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 $[\text{}^{18}\text{O}_2]\text{2}$ and $[\text{}^{17}\text{O}_2]\text{2}$.

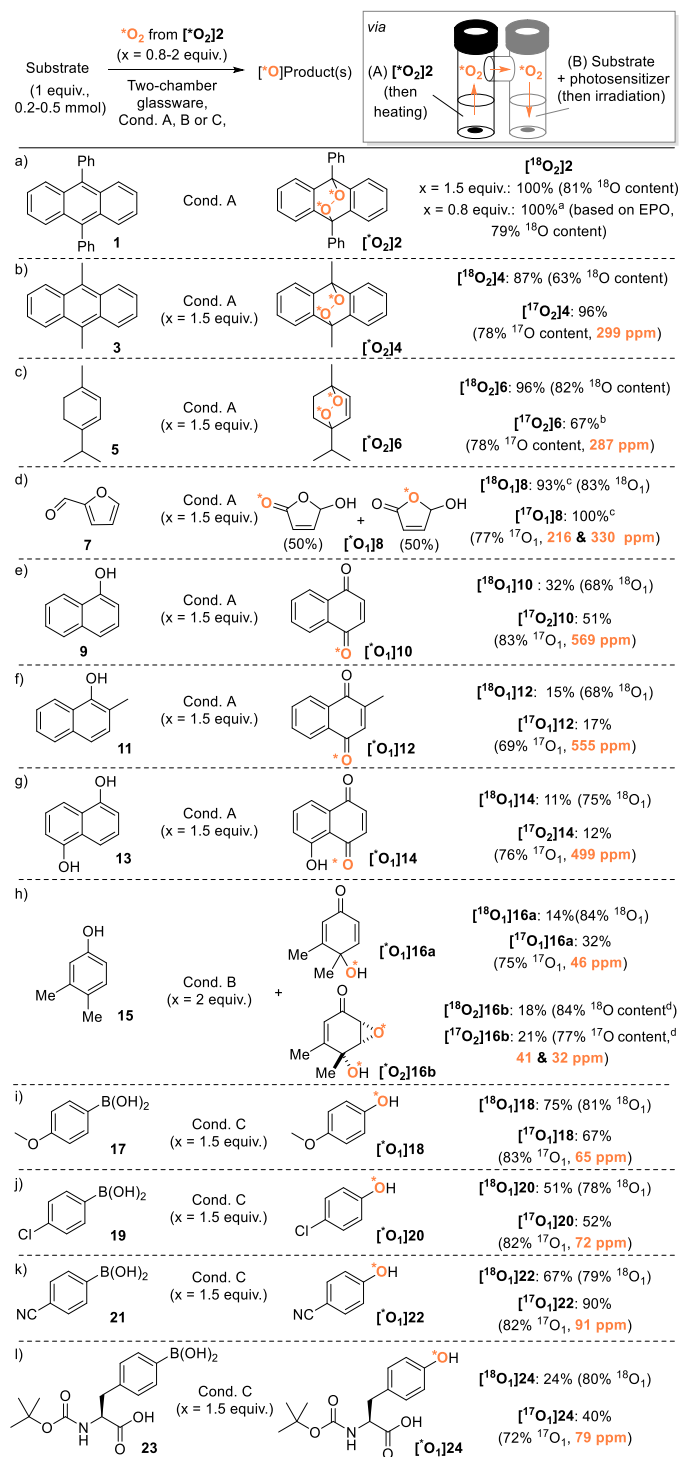


Notably, endoperoxides scaffolds (EPO) are known to be precursors of singlet oxygen,^{11,12,13} and among the various possibilities tested, endoperoxide **2** was selected as the most promising structure. Indeed, it possessed both the ability to generate dioxygen quantitatively when heated over 100°C,¹⁴ 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 $[\text{}^{18}\text{O}_2]\text{2}$ and $[\text{}^{17}\text{O}_2]\text{2}$ were obtained in almost quantitative yields by reacting the commercially available 9,10-diphenylanthracene **1** with photosensitized singlet $[\text{}^*\text{O}_2]$ dioxygen 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 dioxygen provided the desired EPOs with high isotopic enrichments of 84% and 82% for $[\text{}^{18}\text{O}_2]\text{2}$ and $[\text{}^{17}\text{O}_2]\text{2}$, respectively.¹⁵ 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.¹⁶ Firstly, the efficiency of the $^*\text{O}_2$ generation & transfer was investigated by adding 1.5 equiv. of $[\text{}^{18}\text{O}_2]\text{2}$ 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 N_2 by freeze-and-pump cycles, chamber A was heated over 150°C for 3 min.¹⁷ Then, chamber B was irradiated at r.t. for 66 h hours under red light (660 nm LEDs), and $[\text{}^{18}\text{O}_2]\text{2}$ 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 $[\text{}^{18}\text{O}_2]\text{2}$ indicated minimal isotopic losses (Scheme 2, a).¹⁸ Switching to a limiting amount of starting $[\text{}^{18}\text{O}_2]\text{2}$ in chamber A also gave in chamber B $[\text{}^{18}\text{O}_2]\text{2}$ 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 dioxygen was explored, and various substrates were engaged with only a slight excess of $[\text{}^*\text{O}_2]$ endoperoxides $[\text{}^{18}\text{O}_2]\text{2}$ or $[\text{}^{17}\text{O}_2]\text{2}$ (1.5 to 2 equiv.). When 9,10-dimethylantracene **3** was employed as starting material in conditions A at -10°C, the desired $[\text{}^{18}\text{O}_2]\text{4}$ and $[\text{}^{17}\text{O}_2]\text{4}$ 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 $[\text{}^*\text{O}_2]$ ascaridole (an anthelmintic natural product) was investigated by 1,4-cycloaddition of α -terpinene **5** with $^*\text{O}$ -labeled singlet oxygen.¹⁹ In this case, $[\text{}^{18}\text{O}_2]\text{6}$ & $[\text{}^{17}\text{O}_2]\text{6}$ 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 $[\text{}^{18}\text{O}_2]\text{2}$ or $[\text{}^{17}\text{O}_2]\text{2}$, and 5-hydroxy-2(5*H*)-furanone²⁰ 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}O NMR spectrum of [$^{17}\text{O}_1$]**8**, revealed the presence of two signals with identical integrations. As previously described in the literature,²¹ 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.¹⁶ 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)²² [$^*\text{O}_1$]**12** and juglone²³ [$^*\text{O}_1$]**14** with high $^*\text{O}$ -enrichments (68-76%, Scheme 2, f & g). Moreover, ^{13}C NMR demonstrated that the desired carbonyl possessed the sole ^{18}O -labeled atom in [$^{18}\text{O}_1$]**12** and [$^{18}\text{O}_1$]**14**.¹⁶

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,²⁴ 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}O NMR of [$^{17}\text{O}_1$]**16a** confirmed the unique labeling at the hydroxyl group. On the other hand, despite an overall ^{18}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 $\text{C}_8\text{H}_{10}^{16}\text{O}_3$.¹⁶ 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**,¹⁶ 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}O NMR experiment revealed that the two signals of the hydroxy and epoxy groups had equivalent integrations.¹⁶ 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.^{24c} Finally, the photooxidation of boronic acids into phenols²⁵ 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 O_2 even at a low partial pressure. Moreover, these conditions were also applied to prepare in one-step [η - $^{17}\text{O}_1$]-(*S*)-*N*-Boc-tyrosine **24** in 40% yield and 72% of ^{17}O -labeling at the phenol position (Scheme 2, l), which, after *N*-Boc-deprotection, can be used to study protein structures by ^{17}O NMR.²⁶

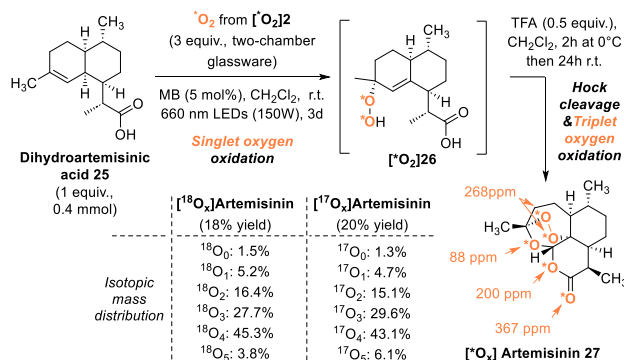
Scheme 2: Oxidations of various substrates by near-stoichiometric amounts of *O_2 produced *ex situ* from EPO [$^{18}O_2$]2 or [$^{17}O_2$]2.



General protocol: [*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 N_2 using freeze-and-pump cycles. Chamber A was heated over $150^\circ C$ for 3 min, then chamber B was irradiated by a suitable light. Conditions A: methylene blue (5 mol%), CH_2Cl_2 or CH_3OH , r.t. or $-10^\circ C$, 660 nm LEDs (150W), 2 d. Conditions B: Cs_2CO_3 (15 mol%), rose bengal (1 mol%), MeOH, r.t., 525 nm LEDs (40W), 36-48h. Conditions C: NEt_3 (40 mol%), rose bengal (5 mol%), EtOH, r.t., 525 nm LEDs (40W), 36h. Isotopic enrichments determined by mass analyses. $\delta(^{17}O)$ in orange. See SI for detailed conditions. ^aIsolated in mixture with **1**. ^bisolated in mixture with 16% of *p*-cymene. ^c5-hydroxy-2(5H)-furanone [*O]8 converted partially converted into [*O]-*trans*- β -formylacrylic acid after isolation ^dCalculated on the *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 compound²⁷ that can be obtained from dihydroartemisinic acid **25** by two consecutive dioxygen oxidations involving a postulated intermediary Hock cleavage.²⁸ In this context, compound **25** was placed in CH₂Cl₂ under N₂ 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 [^{*}O₂]**2** (Scheme 3). After thermal release of ¹⁸O₂ or ¹⁷O₂, chamber B was irradiated under red light at r.t. for three days to enable the desired ene-reaction that formed *in situ* endoperoxide [^{*}O₂]**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 ^{*}O-labeled triplet oxygen in the two-chamber glassware, and spontaneous cyclization occurred to give the desired ^{*}O-labeled versions of artemisinin [¹⁸O_x]**27** and [¹⁷O_x]**27** in decent isolated yields of 18% and 20%, respectively.

Scheme 3: Synthesis of [^{*}O_x]Artemisinin **27 by one-pot double oxidation of dihydroartemisinic acid **25** using ^{*}O₂ produced *ex situ* from 3 equivalents of [¹⁸O₂]**2** or [¹⁷O₂]**2**.**

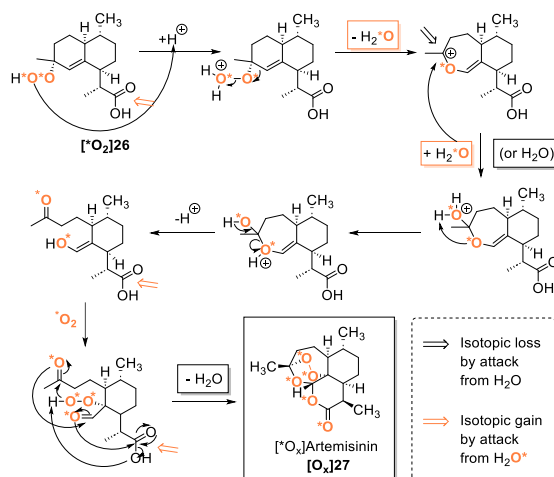


MB: methylene blue. Isotopic distribution determined by mass analyses. $\delta(^{17}\text{O})$ in orange. See SI for exact experimental conditions.

In both cases, mass analyses revealed a mixture of isotopic formulas ranging from zero to five oxygen atoms labeled, yet the expected [^{*}O₄]**27** was the main specie detected in 43% to 45%. Detailed examination of the isotopic mass distribution for the ¹⁸O-labeled artemisinin showed that summing the proportions for [¹⁸O₄]**27** & [¹⁸O₃]**27** (= 73%) and [¹⁸O₂]**27** & [¹⁸O₁]**27** (= 22%) gave values close to the expected results (i.e. 71% of [¹⁸O₄]**27**, 26% of [¹⁸O₂]**27** and 3% of [¹⁸O₀]**27**). This suggested that one ^{*}O-labeled atom was lost partially during the double oxidation process. These results confirmed the Hock mechanism (Scheme 4), which involved the ^{*}O-^{*}O bond cleavage of [^{*}O₂]**26** *via* protonation of the terminal oxygen of the peroxide and consecutive [^{*}O]H₂O departure. Indeed, the water molecule is recaptured in these case by the carbocation issued from the vinyl migration. Thus, as unlabeled H₂O is in the final cyclization cascade, competition occurred at this stage between [^{*}O]H₂O and [¹⁶O]H₂O to trap the carbocation, which decreased partially from one the number of ^{*}O-labeled atoms in the final [^{*}O_x]artemisinin **27**. Moreover, the unreacted ^{*}O-labeled water generated was able to freely exchange with the ^{*}O-atoms of the carboxylic acid until the cyclization step, which explains the small amounts of [¹⁸O₅]**27** detected (3.8%, Scheme 3). Finally, the ¹⁷O NMR spectra of [¹⁷O_x]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 ^{*}O-atoms in the C=O bond.¹⁶

To conclude, easy-to-handle EPOs [¹⁸O₂]**2** and [¹⁷O₂]**2** were synthesized and used to produce near-stoichiometric amounts of ^{*}O₂ *ex situ* in a two-chamber glassware. This strategy allowed the ¹⁸O- and ¹⁷O-labeling of various compounds on small scales with very high isotopic enrichments and at affordable costs. Particularly, analyzing the [^{*}O]products by mass spectrometry and ¹⁷O NMR provided valuable information on the chemical mechanisms involved. Thus, we believe that this method will bring significant benefits for studies on oxidations with O₂.

Scheme 4: Proposed mechanism for the formation of [^xO_x]artemisinin from peroxide [^xO₂]26



ASSOCIATED CONTENT

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Supporting Information

General experimental procedures and analysis/spectroscopic data for new compounds (¹H NMR, ¹³C NMR, ¹⁷O 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).

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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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REFERENCES

- (1) Lloyd-Jones, G.C.; Muñoz, M. P.; Isotopic labelling in the study of organic and organometallic mechanism and structure: an account *J. Label. Compd. Radiopharm.*, **2007**, *50*, 1072-1087.
- (2) (a) Gerothanassis, I. P. Oxygen-17 NMR spectroscopy: Basic principles and applications (Part I) *Prog. Nucl. Magn. Reson. Spectrosc.*, **2010**, *56*, 95-197; (b) Gerothanassis, I. P. Oxygen-17 NMR spectroscopy: Basic principles and applications (Part II) *Prog. Nucl. Magn. Reson. Spectrosc.*, **2010**, *57*, 1-110.
- (3) For a review, see: Theodorou, V.; Skobridis, K.; Alivertis, D.; Gerothanassis, I. P. Synthetic methodologies in organic chemistry involving incorporation of [¹⁷O] and [¹⁸O] isotopes *J. Label. Compd. Radiopharm.*, **2014**, *57*, 481-508.
- (4) For selected recent examples, see: (a) Lloyd-Jones, G. C.; Purser, S. A Metathesis Approach to Volatile Olefins: Synthesis of ¹⁸O-Allyl Alcohol *Chem. Eur. J.*, **2011**, *17*, 4724-4726; (b) Gatenyo, J.; Vints, I.; Rozen, S. The first general route for efficient synthesis of ¹⁸O labelled alcohols using the HOF·CH₃CN complex *Chem. Commun.*, **2013**, *49*, 7379-7381; (c) Beddoe, R. H.; Edwards, D. C.; Goodman, L.; Sneddon, H. F.; Denton, R. M. Synthesis of ¹⁸O-labelled alcohols from unlabelled alcohols *Chem.*

- Commun.*, **2020**, *56*, 6480-6483; Doiuchi, D.; Uchida, T. Catalytic Highly Regioselective C–H Oxygenation Using Water as the Oxygen Source: Preparation of $^{17}\text{O}/^{18}\text{O}$ -Isotope-Labeled Compounds *Org. Lett.*, **2021**, *23*, 7301-7305; (d) Yang, Y.; Han, B.; Dong, F.; Lv, J.; Lu, H.; Sun, Y.; Lei, Z.; Yang, Z.; Ma, H. A Cost-Effective Way to Produce Gram-Scale ^{18}O -Labeled Aromatic Aldehydes *Org. Lett.*, **2022**, *24*, 4409-4414; (e) Katagiri, K.; Kuriyama, M.; Yamamoto, K.; Demizu, Y.; Onomura, O. Organocatalytic Synthesis of Phenols from Diaryliodonium Salts with Water under Metal-Free Conditions *Org. Lett.*, **2022**, *24*, 5149-5154.
- (5) For examples on the use of mechanochemistry for ^{17}O -labeling, see: (a) Métro, T.-X.; Gervais, C.; Martinez, A.; Bonhomme, C.; Laurencin, D. Unleashing the Potential of ^{17}O NMR Spectroscopy Using Mechanochemistry *Angew. Chem. Int. Ed.*, **2017**, *56*, 6803-6807; *Angew. Chem.*, 2017, **129**, 6907-6911; (b) Špačková, J.; Fabra, C.; Mitteleite, S.; Gaillard, E.; Chen, C.-H.; Cazals, G.; Lebrun, A.; Saad, S.; Berthomieu, D.; Chen, K.; Gan, Z.; Gervais, C.; Métro, T.-X.; Laurencin, D. Unveiling the Structure and Reactivity of Fatty-Acid Based (Nano)materials Thanks to Efficient and Scalable ^{17}O and ^{18}O -Isotopic Labeling Schemes *J. Am. Chem. Soc.*, **2020**, *142*, 21068-210181; (c) Špačková, J.; Fabra, C.; Cazals, G.; Hubert-Roux, M.; Schmitz-Afonso, I.; Goldberga, I.; Berthomieu, D.; Lebrun, A.; Métro, T.-X.; Laurencin, D. Cost-efficient and user-friendly $^{17}\text{O}/^{18}\text{O}$ labeling procedures of fatty acids using mechanochemistry *Chem. Commun.*, **2021**, *57*, 6812-6815; (d) Špačková, J.; Goldberga, I.; Yadav, R.; Cazals, G.; Lebrun, A.; Verdié, P.; Métro, T.-X.; Laurencin, D. Fast and Cost-Efficient ^{17}O -Isotopic Labeling of Carboxylic Groups in Biomolecules: From Free Amino Acids to Peptide Chains *Chem. Eur. J.*, **2023**, e202203014.
 - (6) For representative examples, see: (a) Kabalka, G. W.; Reed, T. J.; Kunda, S. A. Synthesis of Oxygen-17 Labeled Alcohols Via Organoborane Reactions *Synth. Commun.*, **1983**, *13*, 737-740; (b) Mohnhaupt, M.; Hagemann, H.; Perler, J.-P.; Bill, H.; Boukouvalas, J.; Rossier, J.-C.; Jefford, C. W. A Vibrational Study of Some 1,2,4-Trioxanes *Helv. Chim. Acta*, **1988**, *71*, 992-999; (c) Carney, J. M.; Hammer, R. J.; Hulse, M.; Lomas, C. M.; Miyashiro, D. Microphotochemistry Using 5-mm Light-Emitting Diodes: Energy-Efficient Photooxidations *Synthesis*, **2012**, *44*, 2560-2566; (d) Pang, X.; Chen, C.; Ji, H.; Che, Y.; Ma, W.; Zhao, J. Unraveling the Photocatalytic Mechanisms on TiO_2 Surfaces Using the Oxygen-18 Isotopic Label Technique *Molecules*, **2014**, *19*, 16291-16311; (e) Zhang, X.; Wu, G.; Gao, W.; Ding, J.; Huang, X.; Liu, M.; Wu, H. Synergistic Photo-Copper-Catalyzed Hydroxylation of (Hetero)aryl Halides with Molecular Oxygen *Org. Lett.*, **2018**, *20*, 708-711.
 - (7) Examples of prices in 2023: 1050 € for 1 L of 99% $^{18}\text{O}_2$ (i.e. 11 €/mmol of ^{18}O), and 5426 € for 1 L of 90% $^{17}\text{O}_2$ (i.e. 66€/mmol of ^{17}O).
 - (8) (a) Hermange, P.; Lindhardt, A. T.; Taaning, R. H.; Bjerglund, K.; Lupp, D.; Skrydstrup, T. Ex Situ Generation of Stoichiometric and Substoichiometric ^{12}CO and ^{13}CO and Its Efficient Incorporation in Palladium Catalyzed Aminocarbonylations *J. Am. Chem. Soc.*, **2011**, *133*, 6061-6071; (b) Friis, S. D.; Lindhardt, A. T.; Skrydstrup, T.; The Development and Application of Two-Chamber Reactors and Carbon Monoxide Precursors for Safe Carbonylation Reactions *Acc. Chem. Res.*, **2016**, *49*, 594-605; (c) Ravn, A. K.; Johansen, M. B.; Skrydstrup, T. Controlled Release of Reactive Gases: A Tale of Taming Carbon Monoxide *ChemPlusChem*, **2020**, *85*, 1529-1533.
 - (9) Modvig, A.; Andersen, T. L.; Taaning, R. H.; Lindhardt, A. T.; Skrydstrup, T. Two-Chamber Hydrogen Generation and Application: Access to Pressurized Deuterium Gas *J. Org. Chem.*, **2014**, *79*, 5861-5868.
 - (10) Min, G. K.; Bjerglund, K.; Kramer, S.; Gøsgisig, T. M.; Lindhardt, A. T.; Skrydstrup, T. Generation of Stoichiometric Ethylene and Isotopic Derivatives and Application in Transition-Metal-Catalyzed Vinylation and Enyne Metathesis *Chem. Eur. J.*, **2013**, *19*, 17603-17607.
 - (11) For reviews on $^1\text{O}_2$, see: (a) Frimer, A. A. The Reaction of Singlet Oxygen with Olefins: The Question of Mechanism *Chem. Rev.*, **1979**, *79*, 359-387; (b) Ogilby, P. R. Singlet oxygen: there is indeed something new under the sun *Chem. Soc. Rev.*, **2010**, *39*, 3181-3209; (c) Montagnon, T.; Kalaitzakis, D.; Triantafyllakis, M.; Stratakis, M.; Vassilikogiannakis, G. Furans and singlet oxygen – why there is more to come from this powerful partnership *Chem. Commun.*, **2014**, *50*, 15480-15498; (d) Ghogare, A. A.; Greer, A. Using Singlet Oxygen to Synthesize Natural Products and Drugs *Chem. Rev.*, **2016**, *116*, 9994-10034.
 - (12) For reviews on the generation of $^1\text{O}_2$ from EPOs, see: (a) Aubry, J.-M.; Pierlot, C.; Rigaudy, J.; Schmidt, R. Reversible Binding of Oxygen to Aromatic Compounds *Acc. Chem. Res.*, **2003**, *36*, 668-675; (b) Filatov, M. A.; Senge, M. O. Molecular devices based on reversible singlet oxygen binding in optical and photomedical applications *Mol. Syst. Des. Eng.*, **2016**, *1*, 258-272; (c) You, Y. Chemical tools for the generation and detection of singlet oxygen *Org. Biomol. Chem.*, **2018**, *16*, 4044-4060; (d) Fudickar, W.; Linker, T. Release of Singlet Oxygen from Organic Peroxides under Mild Conditions *ChemPhotoChem*, **2018**, *2*, 548-558.
 - (13) For the *in situ* use of ^{18}O -labeled endoperoxides for mechanistic studies on singlet oxygen oxidations, see: (a) Martinez, G. R.; Ravanat, J.-L.; Medeiros, M. H. G.; Cadet, J.; Di Mascio, P. Synthesis of a Naphthalene Endoperoxide as a Source of ^{18}O -labeled Singlet Oxygen for Mechanistic Studies *J. Am. Chem. Soc.*, **2000**, *122*, 10212-10213; (b) Di Mascio, P.; Martinez, G. R.; Miyamoto, S.; Ronsein, G. E.; Medeiros, M. H. G.; Cadet, J. Singlet molecular oxygen: Düsseldorf - Sao Paulo, the Brazilian connection *Arch. Biochem. Biophys.*, **2016**, *595*, 161-175; (c) Di Mascio, P.; Martinez, G. R.; Miyamoto, S.; Ronsein, G. E.; Medeiros, M. H. G.; Cadet, J. Singlet Molecular Oxygen Reactions with Nucleic Acids, Lipids, and Proteins *Chem. Rev.*, **2019**, *119*, 2043-2086.
 - (14) (a) Wasserman, H. H.; Scheffer, J. R.; Cooper, J. L. Singlet Oxygen Reactions with 9,10-Diphenylanthracene Peroxide *J. Am. Chem. Soc.*, **1972**, *94*, 4991-4996; (b) Turro, N. J.; Chow, M.-F.; Rigaudy, J. Thermolysis of Anthracene Endoperoxides. Concerted vs. Diradical Mechanisms. Microscopic Reversibility in Endothermic Chemiluminescent Reactions *J. Am. Chem. Soc.*, **1979**, *101*, 1300-1302; (c) Turro, N. J.; Chow, M.-F.; Rigaudy, J. Mechanism of Thermolysis of Endoperoxides of Aromatic Compounds. Activation Parameters, Magnetic Field, and Magnetic Isotope Effects *J. Am. Chem. Soc.*, **1981**, *103*, 7218-7224.
 - (15) The isotopic loss was dependent from the degassing time of the solvent. See SI for details.

- (16) See SI for details.
- (17) **EPO 2** was never described as potentially explosive and it possesses a very low “Active oxygen content“ (<5), which should discard any safety issues of this protocol on millimolar scales using appropriate safety equipment. For general safety regards concerning peroxides, see: Dussault, P., "Working with organic peroxides in the academic lab", *Organic Peroxides: Safety Issues*, **2018**, doi:10.13014/K2GT5KCJ.
- (18) No reaction was observed in chamber B without irradiation, indicating that singlet dioxygen generated from [¹⁸O₂]**2** in chamber A was fully de-excited to triplet dioxygen upon diffusion to the other chamber.
- (19) For selected recent syntheses of ascaridole by flow chemistry, see: (a) Radjagobalou, R.; Blanco, J.-F.; Dechy-Cabaret, O.; Oelgemöller, M.; Loubière, K. Photooxygenation in an advanced led-driven flow reactor module: Experimental investigations and modelling *Chem. Eng. Process.*, **2018**, *130*, 214-228; (b) Radjagobalou, R.; Blanco, J.-F.; Petrizza, L.; Le Behec, M.; Dechy-Cabaret, O.; Lacombe, S.; Save, M.; Loubiere, K. Efficient Photooxygenation Process of Biosourced α -Terpinene by Combining Controlled LED-Driven Flow Photochemistry and Rose Bengal-Anchored Polymer Colloids *ACS Sustain. Chem. Eng.*, **2020**, *8*, 18568-18576.
- (20) Morita, Y.; Tokuyama, H.; Fukuyama, T. Stereocontrolled Total Synthesis of (-)-Kainic Acid. Regio- and Stereoselective Lithiation of Pyrrolidine Ring with the (+)-Sparteine Surrogate *Org. Lett.*, **2005**, *7*, 4337-4340.
- (21) Braun, A. M.; Dahn, H.; Gassmann, E.; Gerotheranassis, I.; Jakob, L.; Kateval, J.; Martinez, C. G.; Oliveros, E. (2+4)-Cycloaddition with Singlet Oxygen. ¹⁷O- Investigation of the Reactivity of Furfuryl Alcohol Endoperoxide *Photochem. Photobiol.*, **1999**, *70*, 868-874.
- (22) (a) Daines, A. M.; Payne, R. J.; Humphries, M. E.; Abell, A. D., The Synthesis of Naturally Occurring Vitamin K and Vitamin K Analogues *Curr. Org. Chem.*, **2003**, *7*, 1625-1634; (b) Shearer, M. J.; Newman, P. Recent trends in the metabolism and cell biology of vitamin K with special reference to vitamin K cycling and MK-4 biosynthesis *J. Lipid Res.*, **2014**, *55*, 345-362.
- (23) (a) Blanchard, V.; Asbai, Z.; Cottet, K.; Boissonnat, G.; Port, M.; Amara, Z. Continuous Flow Photo-oxidations Using Supported Photocatalysts on Silica *Org. Process Res. Dev.*, **2020**, *24*, 822-826; (b) dos S. Moreira, C.; Santos, T. B.; Freitas, R. H. C. N.; Pacheco, P. A. F.; da Rocha, D. R. Juglone: A Versatile Natural Platform for Obtaining New Bioactive Compounds *Curr. Top. Med. Chem.*, **2021**, *21*, 2018-2045.
- (24) (a) Péault, L.; Nun, P.; Le Grogne, E.; Coeffard, V. Multicatalytic dearomatization of phenols into epoxyquinols via a photooxygenation process *Chem. Commun.*, **2019**, *55*, 7398-7401; (b) Fischer, J.; Nun, P.; Coeffard, V. Visible-Light-Driven Transformations of Phenols via Energy Transfer Catalysis *Synthesis*, **2020**, *52*, 1617-1624; (c) Péault, L.; Planchat, A.; Nun, P.; Le Grogne, E.; Coeffard, V. Atom Economical Photocatalytic Oxidation of Phenols and Site-Selective Epoxidation Toward Epoxyquinols *J. Org. Chem.*, **2021**, *86*, 18192-18203; (d) Carson, M. C.; Orzolek, B. J.; Kozlowski, M. C. Photocatalytic Synthesis of para-Peroxyquinols: Total Synthesis of (±)-Stemenone and (±)-Parvistilbine B *Org. Lett.*, **2022**, *24*, 7250-7254; (e) Péault, L.; Rochelle, S.; Planchat, A.; Nun, P.; Le Grogne, E.; Coeffard, V. Epoxidation of Electron-Deficient Alkenes Triggered by Visible- Light-Driven Phenol Photooxidation for the Synthesis of Epoxy Dienone Products *Adv. Synth. Catal.*, **2023**, *365*, 194-200.
- (25) (a) Zou, Y.-Q.; Chen, J.-R.; Liu, X.-P.; Lu, L.-Q.; Davis, R. L.; Jørgensen, K. A.; Xiao, W.-J. Highly Efficient Aerobic Oxidative Hydroxylation of Arylboronic Acids: Photoredox Catalysis Using Visible Light *Angew. Chem. Int. Ed.*, **2012**, *51*, 784-788; *Angew. Chem.*, **2012**, *124*, 808-812; (b) Weng, W.-Z.; Liang, H.; Zhang, B. Visible-Light-Mediated Aerobic Oxidation of Organoboron Compounds Using in Situ Generated Hydrogen Peroxide *Org. Lett.*, **2018**, *20*, 4979-4983; (c) Xu, Y.-T.; Li, C.-Y.; Huang, X.-B.; Gao, W.-X.; Zhou, Y.-B.; Liu, M.-C.; Wu, H.-Y. Photoinduced hydroxylation of arylboronic acids with molecular oxygen under photocatalyst-free conditions *Green Chem.*, **2019**, *21*, 4971-4975.
- (26) Lin, B.; I. Hung, B.; Gan, Z.; Chien, P.-H.; Spencer, H. L.; Smith, S. P.; Wu, G. ¹⁷O NMR Studies of Yeast Ubiquitin in Aqueous Solution and in the Solid State *ChemBioChem*, **2021**, *22*, 826-829.
- (27) (a) Brown, G. D.; Sy, L.-K. In vivo transformations of dihydroartemisinin in *Artemisia annua* plants *Tetrahedron*, **2004**, *60*, 1139-1159; (b) Brown, G. D. The Biosynthesis of Artemisinin (Qinghaosu) and the Phytochemistry of *Artemisia annua* L. (Qinghao) *Molecules*, **2010**, *15*, 7603-7698.
- (28) (a) Lévesque, F.; Seeberger, P. H. Continuous-Flow Synthesis of the Anti-Malaria Drug Artemisinin *Angew. Chem. Int. Ed.*, **2012**, *51*, 1706-1709; (b) Kopetzki, D.; Lévesque, F.; Seeberger, P. H. A Continuous-Flow Process for the Synthesis of Artemisinin *Chem. Eur. J.*, **2013**, *19*, 5450-5456; (c) Kong, J.; Yang, Y.; Wang, W.; Cheng, K.; Zhu, P. Artemisinin acid: A promising molecule potentially suitable for the semi-synthesis of artemisinin *RSC Advances*, **2013**, *3*, 7622-7641; (d) Amara, Z.; Bellamy, J. F. B.; Horvath, R.; Miller, S. J.; Beeby, A.; Burgard, A.; Rossen, K.; Poliakov, M.; George, M. W. Applying green chemistry to the photochemical route to artemisinin *Nature Chem.*, **2015**, *7*, 489-495; (e) Triemer, S.; Gilmore, K.; Vu, G. T.; Seeberger, P. H.; Seidel-Morgenstern, A. Literally Green Chemical Synthesis of Artemisinin from Plant Extracts *Angew. Chem. Int. Ed.*, **2018**, *57*, 5525-5528, *Angew. Chem.*, **2018**, *130*, 5623-5626.