

An Efficient Preparation of ^{18}O -Labeled Epoxides

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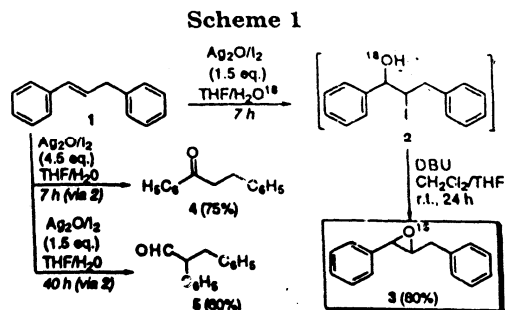
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Isotopically ^{18}O -labeled epoxides have played a critical role in numerous biochemical studies² and there is currently much interest in the role of epoxides and epoxide-converting enzymes in biologically important transformations.³ Consider the numerous recent investigations centered on defining the functions and mechanistic implications of epoxide hydrolases.⁴ Our mechanistic interest in epoxide hydrolase (EH) required ready access to ^{18}O -labeled epoxides and we report here a useful and generally applicable experimental protocol for their preparation from the corresponding olefin and water- ^{18}O . Given the high costs of isotopically enriched starting materials,⁵ it was imperative to develop an ^{18}O -efficient method which proceeds with both high isotopic incorporation and high chemical yield.

Having selected water- ^{18}O as our isotope source, we began by investigating a two-step olefin to halohydrin to epoxide protocol⁶ and were disappointed to find very low levels of ^{18}O -incorporation even using 10 equiv of water- ^{18}O . A number of experiments later, we discovered that the one-pot reaction of *trans*-1,3-diphenylpropene (**1**) with 1.5 equiv of silver(I) oxide plus 1.5 equiv of iodine in THF/water- ^{18}O (60:1, 10 equiv of water- ^{18}O) at room temperature followed by *in situ* addition of 5 equiv of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) for 24 h yielded epoxide- ^{18}O **3** in 80% yield with over 90% ^{18}O -incorporation (Scheme 1).

We were able to intercept and isolate iodohydrin **2** (85%) and thus demonstrate its intermediacy in the preparation of epoxide **3**. In routine preparations of ^{18}O -epoxides, the iodohydrin intermediate was not isolated. Importantly, **2** was obtained as a single diastereomer and a single regioisomer (by $^1\text{H-NMR}$ and GC-MS). Moreover, minor modifications in stoichiometry and reaction time in this step resulted in the clean conversion of



halohydrin **2** to ketone **4** and aldehyde **5**. These reactions were carried out as a single pot synthesis from olefin **1**. Thus, addition of excess silver(I) oxide (4.5 equiv) resulted in base-mediated dehydrohalogenation of intermediate **2** and subsequent tautomerization to ketone **4** (75%). More surprisingly, simply lengthening the reaction time from 7 to 40 h led to a pinacol-type rearrangement of iodohydrin **2** resulting in the formation of aldehyde **5** (60% yield). GC-MS studies with both **4** and **5** established that each carbonyl compound had incorporated an ^{18}O -label (85 and 83% incorporation, respectively). This pinacol process was found to be operative only in those systems where the olefin was phenyl-conjugated. Finally, careful analysis of the **1** \rightarrow **3** reaction mixture (i.e., using 1.5 equiv $\text{Ag}_2\text{O}/\text{I}_2$, 7 h) established that side products **4** and **5** were present in only trace amounts (<5% combined).⁷

The intermediacy of a halohydrin in this epoxidation scheme led us to investigate the possibility of *C=C* *cis/trans*-isomerization with consequent mixtures of epoxide products. The two olefin isomers of 2-heptene were first individually epoxidized with 3-chloroperoxybenzoic acid (MCPBA)⁸ to serve as standards for comparison with our $\text{Ag}_2\text{O}/\text{I}_2/\text{H}_2\text{O}/\text{THF}/\text{DBU}$ epoxidation methodology. In the event, the $\text{Ag}_2\text{O}/\text{I}_2/\text{H}_2\text{O}/\text{THF}/\text{DBU}$ epoxidations of both *cis*-2-heptene and *trans*-2-heptene are stereospecific by capillary GC analysis (Bakerbond DB225 column with flame ionization detector; retention times for *cis*-2,3-epoxyheptane and *trans*-2,3-epoxyheptane are 4.8 and 3.8 min, respectively; less than 0.50% *cis/trans* isomerism of the starting stereochemistry was observed in each case).

The high levels of ^{18}O -incorporation in **1** \rightarrow **3** when 10 equiv of water- ^{18}O (i.e., 195 μL of H_2^{18}O /mmol of **1**) were employed prompted us to investigate the percent of ^{18}O -incorporation as a function of water- ^{18}O equivalents. For these experiments, oleic acid (0.25 mmol) was epoxidized with varying measures of water- ^{18}O . The resulting epoxy stearic acid products were silylated with *N*-methyl-*N*-(*tert*-butyldimethylsilyl)trifluoroacetamide (MTBSTFA) and analyzed by GC/EI-MS (Bakerbond DB17 capillary column) to quantify the percent of ^{18}O -incorporation. The $[\text{M} - (\textit{tert}\text{-butyl})]^+$ fragment peak was selected as the diagnostic signal from which the percent ^{18}O -incorporation was calculated; results from this study are presented in Table 1.

Not surprisingly, the percent of ^{18}O -incorporation decreases as the number of water- ^{18}O equivalents de-

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(5) The current cost of water- ^{18}O (Aldrich) is \$425/g (95 atom % ^{18}O).

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(7) These results with **1** \rightarrow **4/5** are not general since treating oleic acid with increased Ag_2O over extended reaction times did not result in products related to **4** and **5**.

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Table 1. ^{18}O -Incorporation in Oleic Acid Epoxidations

μL of $\text{H}_2^{18}\text{O}/\text{mmol}$	H_2O^{18} , equiv	% chemical yield	% incorporation ^a
195	10.5	95	98.1
150	8.3	96	92.7
100	5.5	95	89.3
50	2.8	92	79.5
25	1.4	84	71.2
10	0.6	50	41.5
0	0	25	0

^a Epoxystearic acid samples were derivatized with MTBSTFA 1 hour prior to analysis by GC/EI-MS. Percent incorporation was calculated from GC/EI-MS data where the monitored mass was the $[\text{M} - (\text{tert-butyl})]^+$ fragment of TBDMS-derivatized samples corrected for natural $[\text{M} + 2]^+$ signals (obtained from epoxy- ^{16}O stearic acid), normal ^{18}O isotopic impurity of the H_2^{18}O , as well as silicon isotopic impurities.

Table 2

entry	olefin	% yield of epoxide
1	<i>trans</i> -2-heptene	88
2	<i>cis</i> -2-heptene	86
3	<i>cis</i> -2-hexen-1-ol	70
4	1-hexen-3-ol	72
5	oleic acid	95
6	cyclohexene	62
7	4-(3-phenyl-1-propenyl)anisole ^{10a}	64
8	4-(3-phenyl-1-propenyl)nitrobenzene ^{10b}	70
9 ^a	<i>cis</i> -stilbene	0
10	<i>trans</i> -stilbene	0

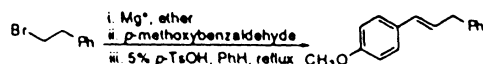
^a *cis*-Stilbene was isomerized to a 55:45 *cis:trans* mixture.

creases. It is remarkable, however, that as few as 1.4 equiv of water- ^{18}O delivers 71.2% ^{18}O -incorporation; that is, 100 mg of oleic acid requires only 8.8(1) μL of water- ^{18}O for 71.2% ^{18}O -incorporation. This result with 1.4 equiv of water- ^{18}O is particularly noteworthy since the experiment with no added water (THF dried by distillation from sodium/benzophenone) still gives a 25% yield of epoxy stearate (note: the source of oxygen in this experiment is either silver oxide or serendipitous H_2O).

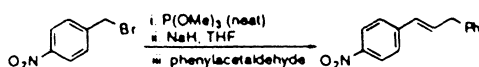
Finally, to establish the scope of this method, a number of olefin to epoxide reactions were investigated in experiments utilizing natural abundance water (Table 2). Entries 1 and 2 were chosen as a probe of *cis/trans*-olefin isomerism (*vide infra*). Entries 3 and 4 were selected to investigate the influence of an allylic hydroxyl on the reaction and, with the addition of 5 equiv of DBU, we were pleased to see no Payne rearrangement⁹ crossover in either system. The oleic acid entry (no. 5) represents the optimized reaction, and it is noteworthy that epoxy stearic acid is obtained essentially pure ($^1\text{H-NMR}$) without chromatographic purification. The *p*-methoxy and the *p*-nitro analogs of 1,3-diphenylpropene (entries 7 and 8) were investigated to probe how inductive effects would

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(10) (a) 4-(3-Phenyl-1-propenyl)anisole (Table 1, entry no. 7) was prepared by dehydrating (5% *p*-TsOH, PhH, reflux) the alcohol obtained by condensation of $\text{PhCH}_2\text{CH}_2\text{MgBr}$ with *p*-methoxybenzaldehyde.



(b) 4-(3-Phenyl-1-propenyl)nitrobenzene (Table 1, entry no. 8) was synthesized by standard Wittig chemistry from dimethyl (4-nitrobenzyl) phosphonate and phenylacetaldehyde.



effect the epoxidation reaction. Perhaps not surprisingly, both systems suffer reduced yields, the former as a consequence of product instability and the latter as a consequence of reduced reactivity in halohydrin formation. Finally, the highly conjugated *cis*- and *trans*-stilbenes proved unreactive and starting material was isolated (note: in the case of *cis*-stilbene, a mixture of *cis*- and *trans*-stilbene was obtained).

In conclusion, these experiments establish that many olefin + $\text{Ag}_2\text{O}/\text{I}_2$ oxidation systems are efficient at scavenging very small amounts of water- ^{18}O and, upon DBU treatment, directing it for stereospecific ^{18}O -epoxidation. Given the high price of water- ^{18}O , this method delivers a mild, cost-effective, and efficient synthesis of ^{18}O -labeled epoxides.¹¹

Experimental Section

All starting materials were purchased from Aldrich, Inc. and used without further purification. The THF used for epoxidation reactions was kept over CaH (\geq one week), refluxed (two days) over sodium/benzophenone, and distilled under dry nitrogen immediately prior to use.

General Epoxidation Method. Water (195 $\mu\text{L}/\text{mmol}$; either ^{16}O or ^{18}O) was added to a dry THF (3 mL) solution of olefin (0.25 mmol) at room temperature. After 5 min, iodine (0.375 mmol) and silver oxide (0.375 mmol) were introduced in one portion and the reaction was stirred under N_2 at room temperature. After 7 h, the brown color of iodine had disappeared and an orangish-brown AgI precipitate was evident. DBU (1.25 mmol) dissolved in CH_2Cl_2 (10 mL) was added to the stirred solution and the precipitate immediately changed to a grayish color. After an additional 5–19 h, the reaction was quenched with 1% H_2SO_4 (5 mL) and was filtered through Celite. The filtrate was diluted with CH_2Cl_2 (20 mL), washed with 1% H_2SO_4 (3 \times 20 mL) and brine (20 mL), dried over anhydrous Na_2SO_4 , and filtered. Solvent removal under reduced pressure delivered the epoxide in generally high purity (as determined by $^1\text{H-NMR}$).¹²

2-Iodo-1,3-diphenyl-1-propanol (2). In order to isolate the iodohydrin, the general epoxidation methodology was followed except that after 6 h the reaction was quenched by Celite filtration of the precipitate (instead of addition of DBU). Ether (30 mL) was added to the filtrate and the organic layer was extracted with 5% $\text{Na}_2\text{S}_2\text{O}_3$ (3 \times , 30 mL), followed by 1% H_2SO_4 (20 mL) and saturated NaCl (20 mL). The organic layer was dried over anhydrous Na_2SO_4 , and solvent was removed under reduced pressure. The product was isolated as a clear oil, which darkened after time (85% yield). The crude product was \approx 95% by $^1\text{H-NMR}$, and attempts at further purification resulted in decomposition: FTIR (neat) 3402, 3081, 3058, 3027, 2923, 1601 cm^{-1} ; $^1\text{H-NMR}$ (300 MHz, CDCl_3) δ 2.65 (1H, bs, D_2O exchangeable), 3.03 (2H, dd), 4.55 (1H, dt), 5.04 (1H, bs), 7.04–7.43 (10H, m); $^{13}\text{C-NMR}$ (75 MHz, CDCl_3) δ 26.8, 39.2, 46.2, 126.4, 126.6, 128.1, 128.2, 128.4, 128.8, 139.4, 140.2; MS (TMS-derivatized) m/z , 395 ($\text{M} - \text{CH}_3$)⁺, 283 ($\text{M} - \text{I}$)⁺, 179 (PhCHOTMS)⁺, 91 (C_7H_7)⁺.

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(11) It is instructive to note that the quantity of water- ^{18}O required as solvent for one EH enzymatic assay^{3*} with unlabeled substrate supplies enough ^{18}O -labeled substrate to run \approx 15 000 enzymatic assays in normal water.

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Supplementary Material Available: ^1H -NMR and ^{13}C -NMR spectra for **2** (2 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.