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

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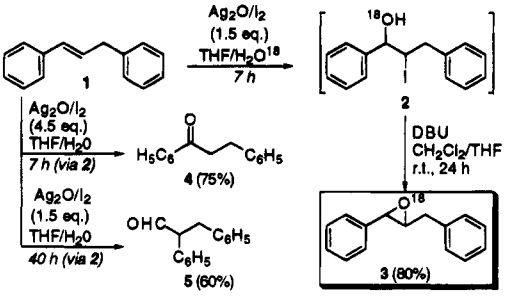
Isotopically ^{18}O-labeled epoxides have played a critical role in numerous biochemical studies[2] and there is currently much interest in the role epoxides and epoxide-converting enzymes play in biologically important transformations.[3] Consider the numerous recent investigations centered on defining 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 protocol[6] 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 ^1H-NMR and GC-MS). Moreover, minor modifications in stoichiometry and reaction time in this step resulted in the clean conversion of

Scheme 1



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** → **3** reaction mixture (i.e., using 1.5 equiv Ag_2O/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 $Ag_2O/I_2/H_2O$/THF/DBU epoxidation methodology. In the event, the $Ag_2O/I_2/H_2O$/THF/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** → **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)triflouroacetamide (MTBSTFA) and analyzed by GC/EI-MS (Bakerbond DB17 capillary column) to quantify the percent of ^{18}O-incorporation. The $[M - (tert\text{-butyl})]^+$ 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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(1) M.J.K. is an NIH RCDA recipient (1989–1994; EC00182).

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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** → **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 $H_2{}^{18}O$/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 $[M - (tert\text{-butyl})]^+$ fragment of TBDMS-derivatized samples corrected for natural $[M + 2]^+$ signals (obtained from epoxy-^{16}O stearic acid), normal ^{16}O isotopic impurity of the $H_2{}^{18}O$, as well as silicon isotopic impurities.

Table 2

entry	olefin	% yield of epoxide
1	*trans*-2-heptene	88
2	*cis*-2-heptene	86
3	*cis*-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]	*cis*-stilbene	0
10	*trans*-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(!) 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 rearrangement[9] 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}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 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 + Ag_2O/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 (≥ one week), refluxed (two days) over sodium/benzophenone, and distilled under dry nitrogen immediately prior to use.

General Epoxidation Method. Water (195 μL/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 a 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 (5mL) and was filtered through Celite. The filtrate was diluted with CH_2Cl_2 (20 mL), washed with 1% H_2SO_4 (3 × 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}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% $Na_2S_2O_3$ (3×, 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 ≈95% by ^{1}H-NMR, and attempts at further purification resulted in decomposition: FTIR (neat) 3402, 3081, 3058, 3027, 2923, 1601 cm^{-1}; ^{1}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}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 $(M - CH_3)^+$, 283 $(M - I)^+$, 179 $(PhCHOTMS)^+$, 91 $(C_7H_7)^+$.

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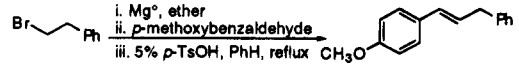
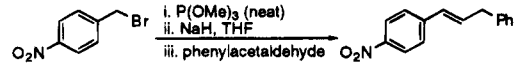
(10) (a) 4-(3-Phenyl-1-propanyl)anisole (Table 1, entry no. 7) was prepared by dehydrating (5% *p*-TsOH, PhH, reflux) the alcohol obtained by condensation of $PhCH_2CH_2MgBr$ with *p*-methoxybenzaldehyde.



(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.



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

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Supplementary Material Available: ^{1}H-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.