

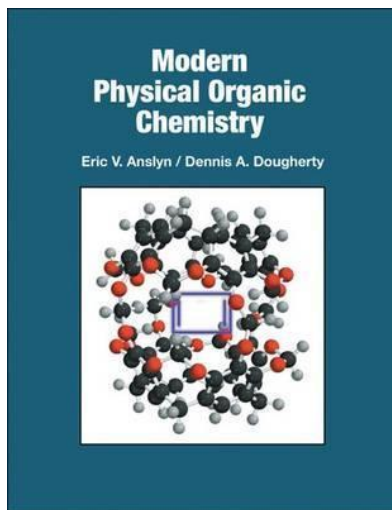
# Kinetic Isotope Effect: Principles and its use in mechanism investigation

**Group Seminar**

05.03.2020

Alexandre Leclair

# Important literature



Modern Physical Organic Chemistry by Eric. V. Anslyn and Dennis A. Dougherty - University Science Books: Sausalito, CA, **2006**  
→ Chapter 8 – Experiments Related to Thermodynamics and Kinetics – 421-441.

Annual Reports on NMR Spectroscopy, Chapter 3 – Application of NMR spectroscopy in Isotope Effects Studies by Stefan Jankowski - (Ed.: G.A. Webb), Academic Press, **2009**, 149–191.



Annual Reports on NMR Spectroscopy  
Volume 68, 2009, Pages 149-191



## CHAPTER 3 - Application of NMR Spectroscopy in Isotope Effects Studies

Stefan Jankowski

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[https://doi.org/10.1016/S0066-4103\(09\)06803-3](https://doi.org/10.1016/S0066-4103(09)06803-3)

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C–H Functionalization

DOI: 10.1002/anie.201107334

## On the Interpretation of Deuterium Kinetic Isotope Effects in C–H Bond Functionalizations by Transition-Metal Complexes\*\*

Eric M. Simmons and John F. Hartwig\*

# CHEMICAL REVIEWS

REVIEW

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Kinetic Isotope Effects in the Study of Organometallic Reaction Mechanisms

Mar Gómez-Gallego\* and Miguel A. Sierra\*

Departamento de Química Orgánica I, Facultad de Química, Universidad Complutense, 28040 Madrid, Spain

E. M. Simmons, J. F. Hartwig, *Angew. Chem. Int. Ed.* **2012**, *51*, 3066-3072.

M. Gomez-Gallego, M. A. Sierra, *Chem. Rev.* **2011**, *111*, 4857-4963

# Table of contents

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- I. Origins of the Kinetic Isotope Effect
- II. Types of Kinetic Isotope Effect
- III. Classical experiments
- IV. KIE measured at natural-abundance
- V. Conclusion and Outlook

# Table of contents

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I. Origins of the Kinetic Isotope Effect

II. Types of Kinetic Isotope Effect

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IV. KIE measured at natural-abundance

V. Conclusion and Outlook

# Origins of the Kinetic Isotope Effect (KIE)

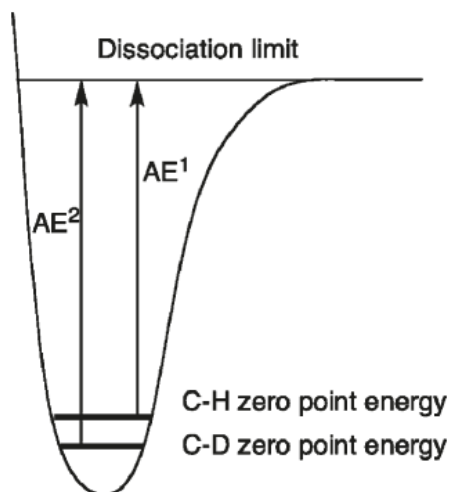
## Origin of isotope effects:

- Difference in **frequencies** of various vibrational modes of a molecule
- No important difference in the potential energy of the system
- However, difference in vibrational states, quantified by the formula:

$$e_n = (n + \frac{1}{2})h\nu, \text{ where } n = 0, 1, 2, \dots$$

- The vibrational modes for bond stretches → dominated by  $n=0$ , with  $e_0 = 1/2h\nu$

**$e_0 = \text{zero-point energy (ZPE)}$**



Morse potential and vibrational energy levels:

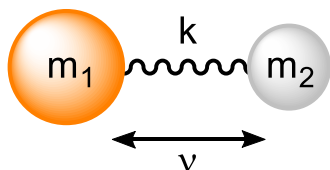
# Origins of the Kinetic Isotope Effect (KIE)

Importance of the reduced mass and force constant:

$$e_n = (n + \frac{1}{2})h\nu, \text{ where } n=0,1,2,\dots$$

Vibration frequency  $\nu$  : 
$$\nu = \frac{1}{2\pi} \sqrt{\frac{k}{m_r}} \quad \longrightarrow \quad \text{Proportional to } \sqrt{\frac{1}{m_r}}$$

With  $m_r$ : reduced mass and  $k$ : force constant



$$m_r = \frac{m_1 m_2}{m_1 + m_2}$$

$m_1$ : "Heavy" atom (C, N, O, ...)

$m_2$ : "Light" atom (H, D, ...)

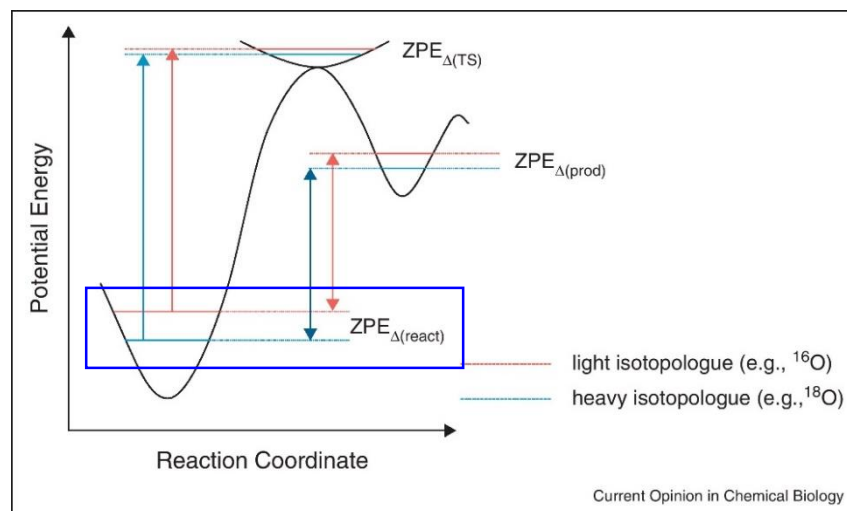
Implications in KIE:

H and D different mass (1.00797 / 2.01410)

So for C-H vs C-D:  $m_{r(\text{C-H})} < m_{r(\text{C-D})}$

$\rightarrow \nu_{\text{C-H}} > \nu_{\text{C-D}}$

$$\text{ZPE}_{(\text{C-H})} > \text{ZPE}_{(\text{C-D})}$$



# Origins of the Kinetic Isotope Effect (KIE)

## Implications in KIE:

$ZPE_{(C-H)} > ZPE_{(C-D)}$   $\longrightarrow$  Homolysis of C-D require more energy = **slower**

Same principle with heavier atom ( $^{12/13}\text{C}$ ,  $^{16/18}\text{O}$ ,  $^{14/15}\text{N}$ , ...)

BUT mass difference smaller  $\rightarrow$  KIE smaller

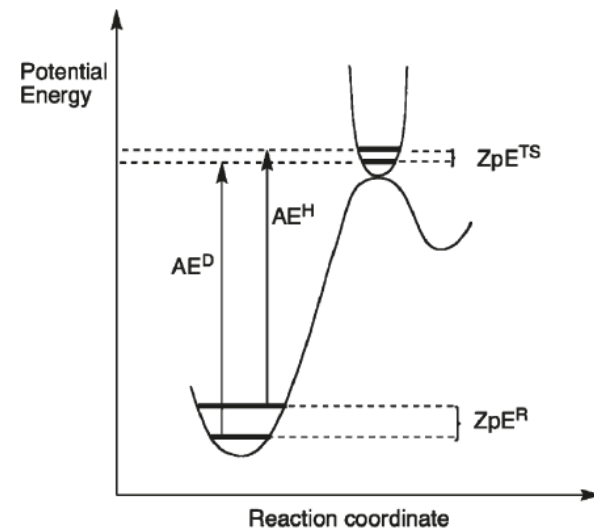
## Magnitude of the observed KIE:

$\Delta ZPE^{\text{transition state}} < \Delta ZPE^{\text{reactants}} \rightarrow \Delta AE_{H/D}$  **important**

Impacted by different factors:

- $\rightarrow$  Vibrations of the bond undergoing reaction
- $\rightarrow$  Geometry of the TS (linear vs non-linear)
- $\rightarrow$  Degree of bond breaking/making
- $\rightarrow$  Position of the TS (exothermic, endothermic, thermoneutral)

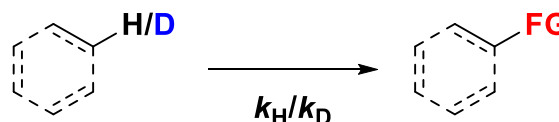
With AE: activation energy



$ZpE^R$  = reactants zero point energy difference  
 $ZpE^{TS}$  = Transition state zero point energy difference

# Types of Kinetic Isotope Effect (KIE)

## Primary Kinetic Isotope Effects:



→ Bond breaking event at the X-H/X-D bond

- If  $k_H/k_D = 1$ : **C-H/D not functionalized during the rate-determining step** (or very small KIE)
- Classical primary KIE:  $1 \ll k_H/k_D \leq 6.5-7^*$  → **The C-H bond functionalization is the rate-determining step\*\***
- Usually in the Transition State (TS): Bond partially broken or new bond start to form

→ **Attenuation of KIE**

\*Maximum  $k_H/k_D \leq 6.5-7$  for a bond 100% broken during the transition state (at  $T=298$  K, with IR (C-H stretch) =  $3000 \text{ cm}^{-1}$ )

\*\* : In parallel experiments, see later in the presentation



# Types of Kinetic Isotope Effect (KIE)

## Primary Kinetic Isotope Effects:

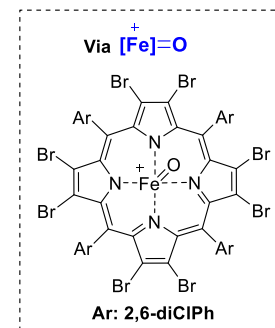
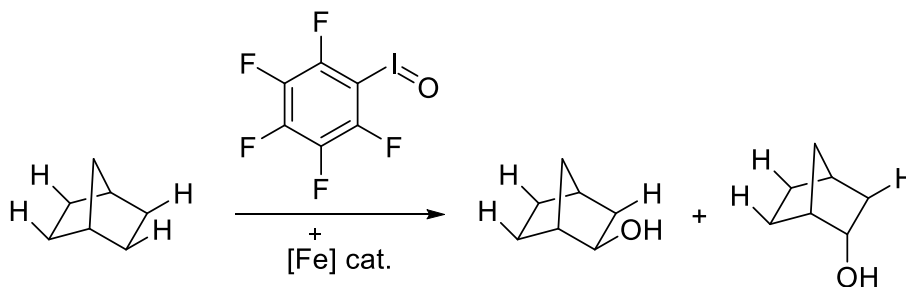


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- Usually in the Transition State (TS): Bond partially broken or new bond start to form

### → Attenuation of KIE

Example: Mechanism of aliphatic hydroxylation by Fe(III) porphyrins (Traylor *et al.*, 1992)



→ Common reaction in biological systems catalyzed by Cytochrome P-450

\*Maximum  $k_H/k_D \leq 6.5-7$  for a bond 100% broken during the transition state (at  $T=298$  K, with IR (C-H stretch) =  $3000$   $\text{cm}^{-1}$ )

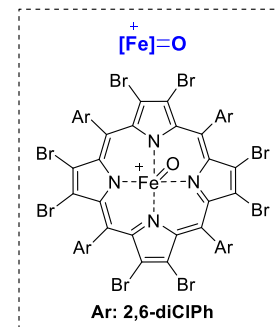
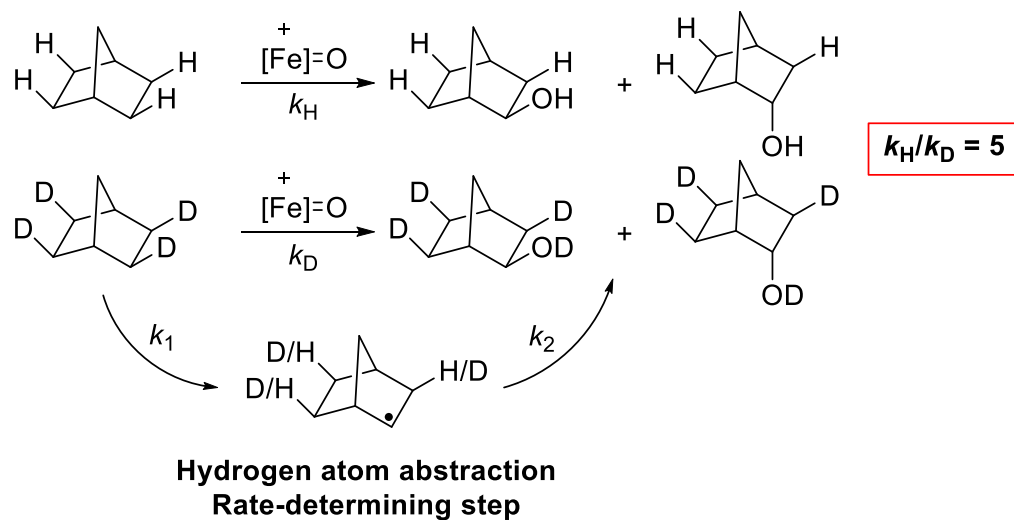
\*\* : In parallel experiments, see later in the presentation

# Types of Kinetic Isotope Effect (KIE)

## Primary Kinetic Isotope Effects:

Example: Mechanism of aliphatic hydroxylation by Fe(III) porphyrins (Traylor *et al.*, 1992)

→ Comparison of the reaction rate with H/D-substrates



Large  $KIE_{H/D}$  + no skeleton rearrangement (no cation):

**Radical cage mechanism**

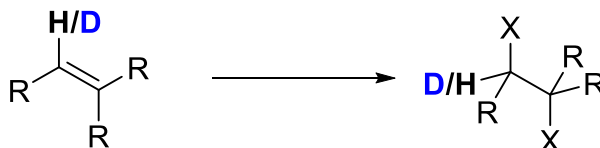
Similar to KIE for the enzyme-catalyzed reaction: Good model

# Types of Kinetic Isotope Effect (KIE)

## Secondary Kinetic Isotope Effects:

→ X-H/X-D not functionalized, remote from the reacting bond

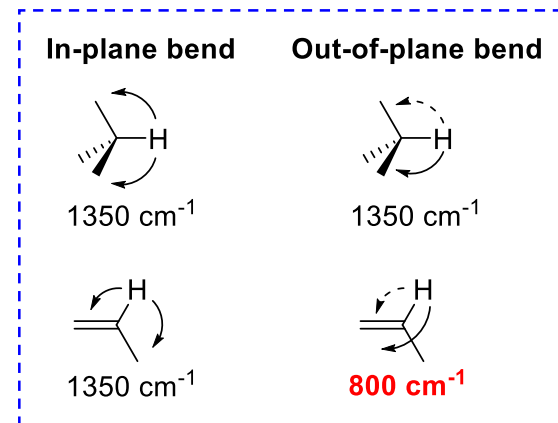
### Via Rehybridization:



→ Difference in force constant (k) for out-of-plane bend

sp<sup>3</sup> → sp<sup>2</sup>: Secondary KIE<sub>H/D</sub> ≈ 1.1-1.2

sp<sup>2</sup> → sp<sup>3</sup>: Inverse KIE<sub>H/D</sub> ≈ 0.8-0.9

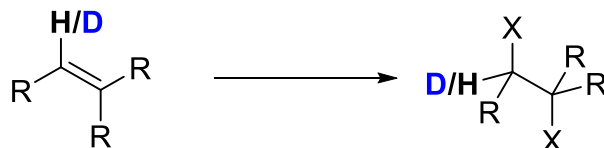


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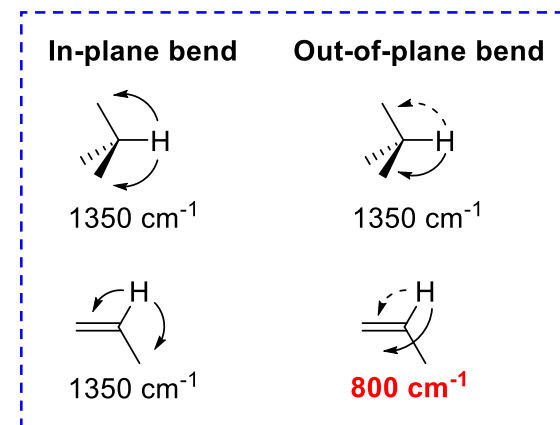
### Via Rehybridization:



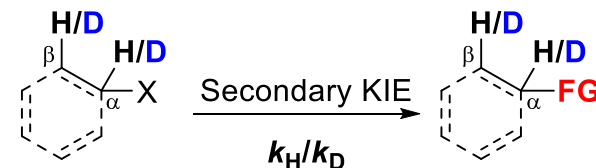
→ Difference in force constant ( $k$ ) for out-of-plane bend

$sp^3 \rightarrow sp^2$ : Secondary  $KIE_{H/D} \approx 1.1-1.2$

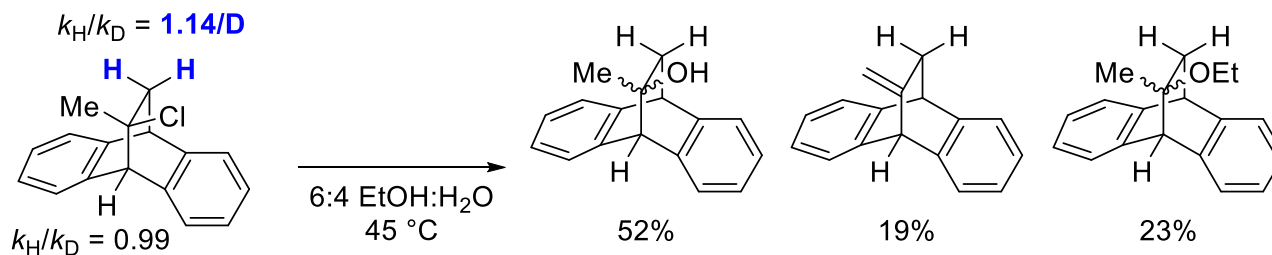
$sp^2 \rightarrow sp^3$ : Inverse  $KIE_{H/D} \approx 0.8-0.9$



### Via electronic effect / hyperconjugation (in the rate-determining step):



→ Example:  $S_N1$  mechanism,  $\beta$ -secondary KIE (Shiner *et al.*, 1963)

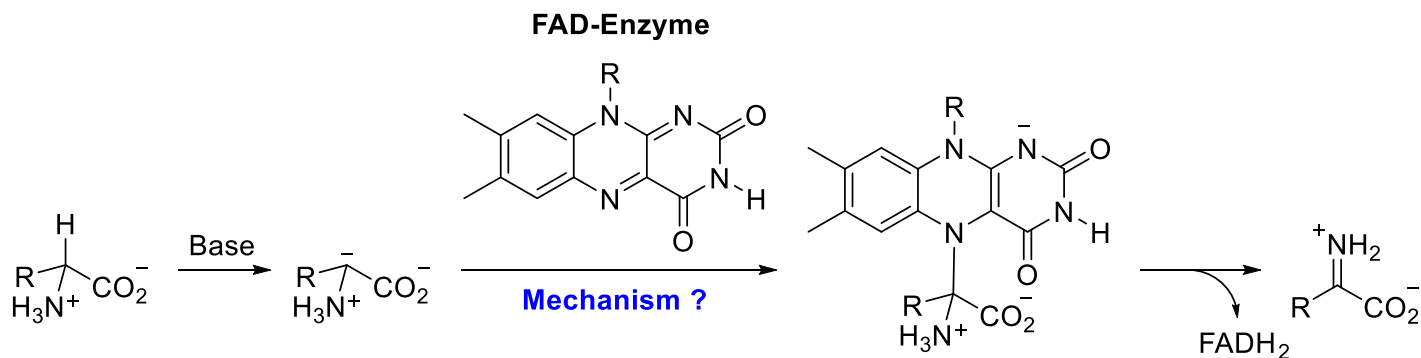


# Types of Kinetic Isotope Effect (KIE)

## Secondary Kinetic Isotope Effects:

Example: Investigation toward D-Amino acid oxidase mechanism (Fitzpatrick *et al.*, 1997)

- Enzyme catalyzing oxidation of amino acids to imino acids using FAD as coenzyme.



- Difficulty to analyze the direct reaction with amino acid carbanion

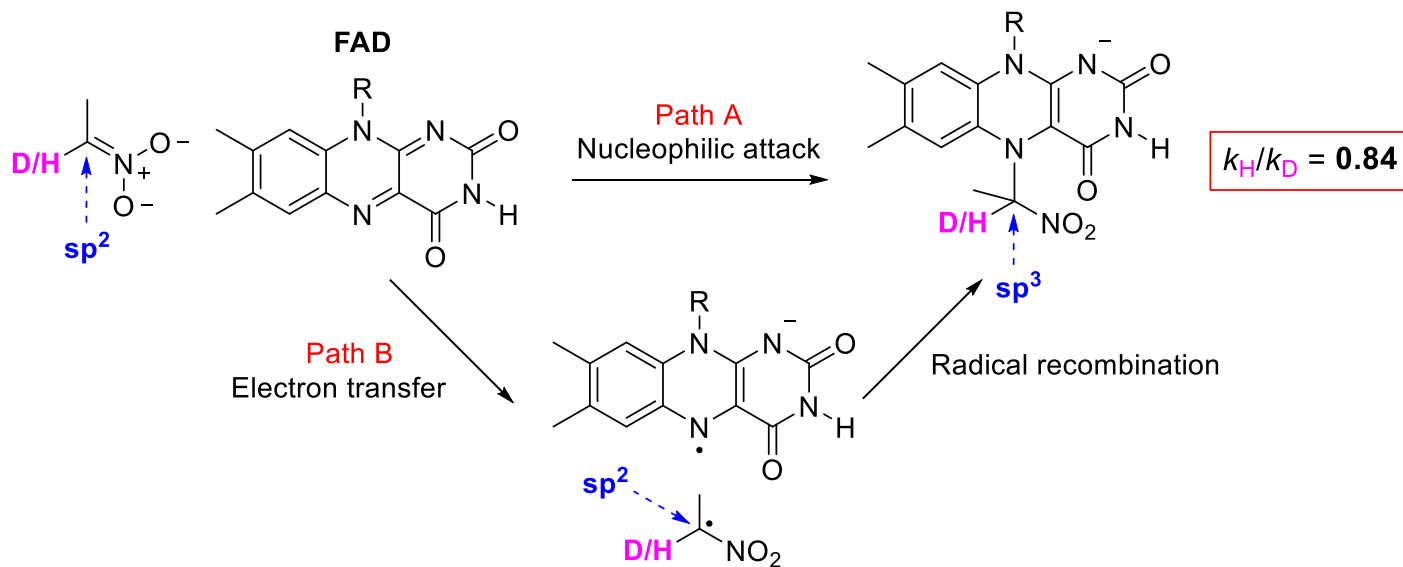
→ Nitroalkane anion used, stable at physiologic pH

➡ **Studies of the secondary kinetic isotope effect at the carbanion position**

# Types of Kinetic Isotope Effect (KIE)

## Secondary Kinetic Isotope Effects:

Example: Investigation toward D-Amino acid oxidase mechanism (Fitzpatrick *et al.*, 1997)



### Hypothesis:

In path A: Change in hybridization from  $sp^2$  to  $sp^3$  → Inverse isotope effect predicted

In path B: No hybridization change → Little or no isotope effect expected

Conclusion:  $k_H/k_D = 0.84$  → Inverse secondary isotope effect: **Support path A**

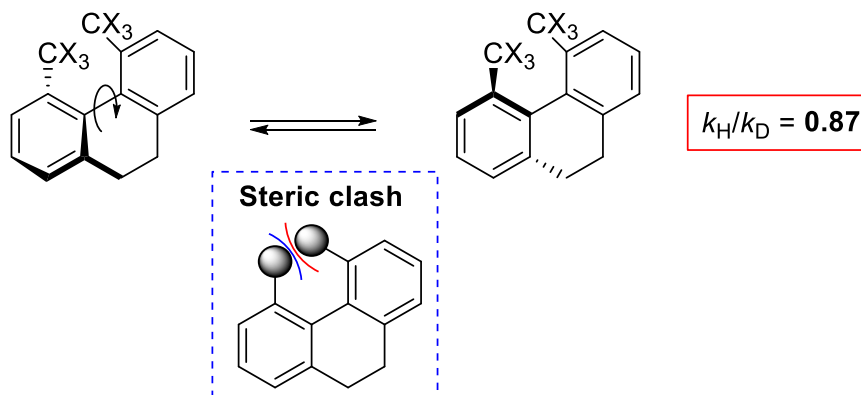
# Types of Kinetic Isotope Effect (KIE)

## Steric Isotope Effects:

- Effective size of H/D important

→ Vibrational amplitude of D smaller: **D appears smaller than H**

Example: Racemization of 9,10-dihydro-4,5-dimethylphenanthrenes (Mislow *et al.*, 1963)

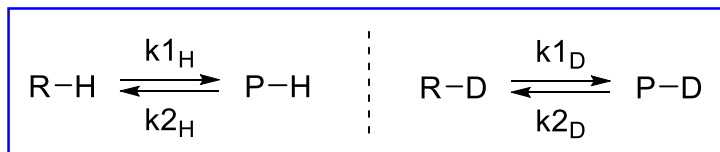


➔  $k_H/k_D = 0.87 \rightarrow$  **Racemization faster with X = D** (D effectively smaller)

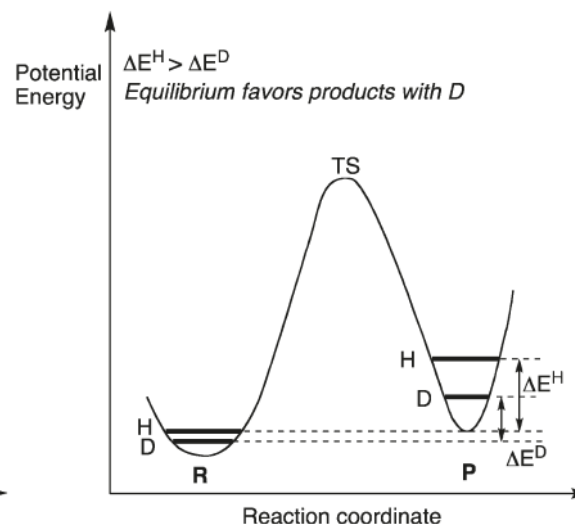
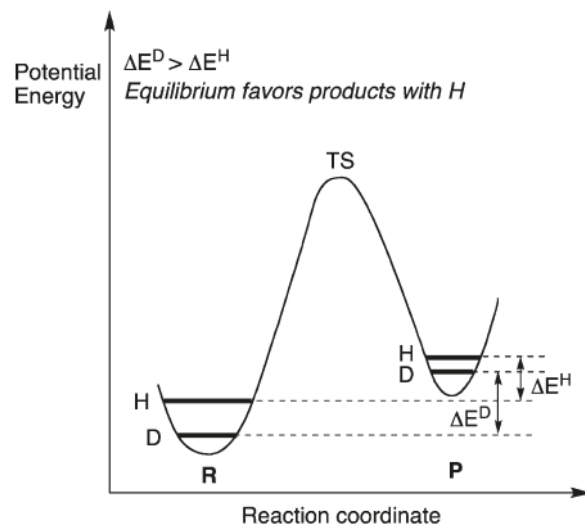
# Types of Kinetic Isotope Effect (KIE)

## Equilibrium Isotope Effects (EIE):

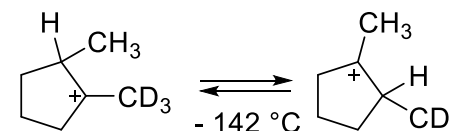
→ In case of reversible reaction, partial fractionation may occur via equilibrium:



$$K_{\text{eq}} = \frac{k_{1\text{H}}/k_{1\text{D}}}{k_{2\text{H}}/k_{2\text{D}}}$$



Example: Carbocation equilibrium involving 1,2-hydride shift  
(Saunders et al., 1977)



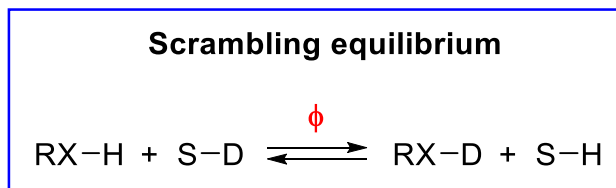
$$K_{\text{eq}} = 1.9$$



# Types of Kinetic Isotope Effect (KIE)

## Solvent Isotope Effects:

- Alcohols O-H, amines N-H, amides N-H, thiols S-H: **readily exchange H/D in D<sub>2</sub>O/MeOD**



→ **Fractionation factor,  $\phi$**        $K_{\text{eq}} = \phi = \frac{[\text{S-H}][\text{X-D}]}{[\text{S-D}][\text{X-H}]}$

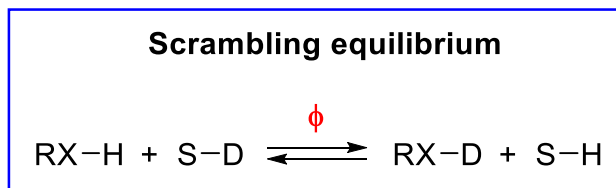
→ Via solvation of the activated complex

→ Via hydrogen transfer

# Types of Kinetic Isotope Effect (KIE)

## Solvent Isotope Effects:

- Alcohols O-H, amines N-H, amides N-H, thiols S-H: **readily exchange H/D in D<sub>2</sub>O/MeOD**

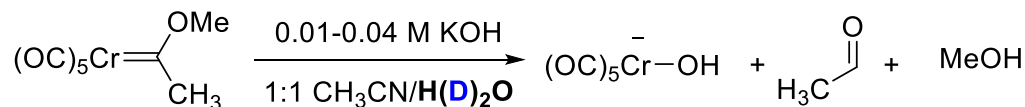


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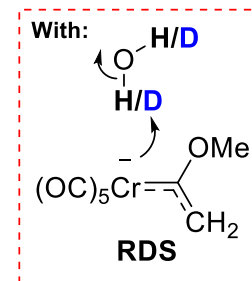
→ Via solvation of the activated complex

→ Via hydrogen transfer

Example: Hydrolysis of Cr-carbene in aqueous acetonitrile (Bernasconi *et al.*, 1995)



$k_{\text{H}_2\text{O}}/k_{\text{D}_2\text{O}} = 3.83-4.15$



→ Solvent isotope effects used for Proton inventories = Nb of H transferred or impacted in the RDS (often used in enzymatic mechanism investigation)

# Types of Kinetic Isotope Effect (KIE)

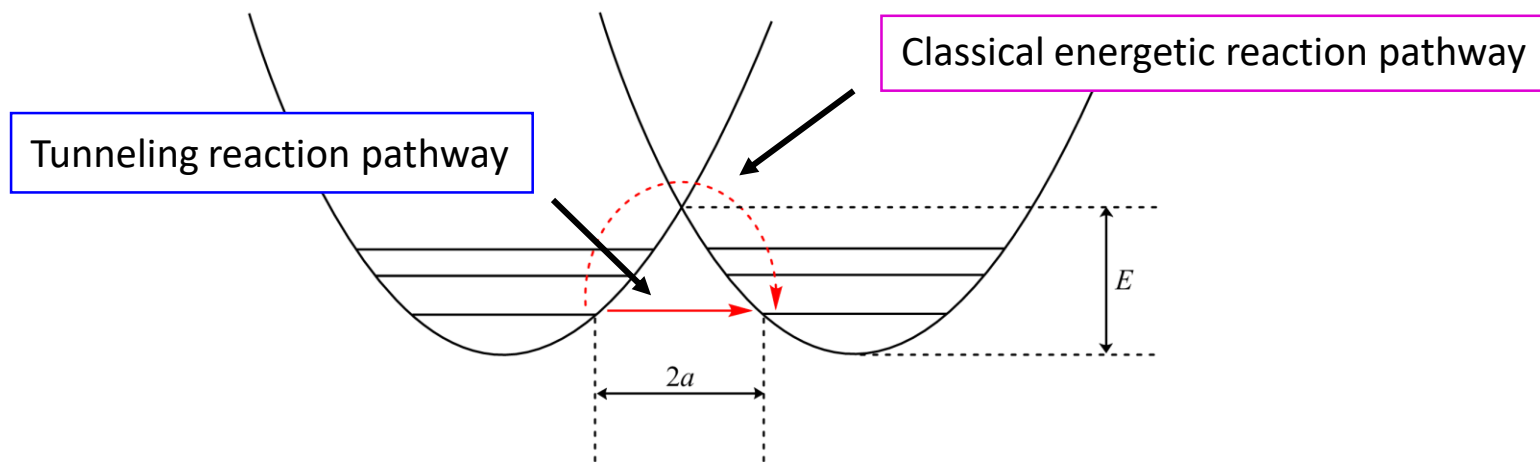
## Tunneling effect:

Sometimes  $KIE_{H/D} > 50$  observed

→ Attributed to **quantum mechanical tunneling**



Quantum mechanical phenomenon where the wave-function for the molecule **penetrates through the energetic barrier** rather than over it.



→ Very common under cryogenic conditions (decrease classical energetic reaction pathways)

➡ One of the simplest analyses of tunneling: **Bell's modification of Arrhenius equation**

# Types of Kinetic Isotope Effect (KIE)

Bell's modification of Arrhenius equation:

$$k = QAe^{-E/RT}$$

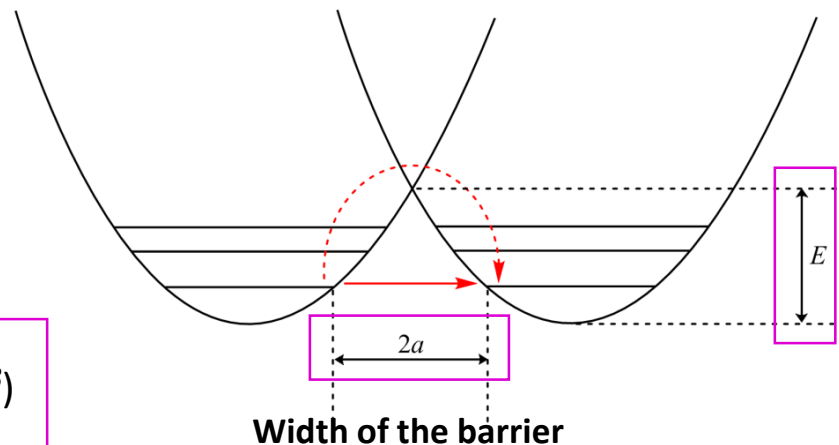
A: Arrhenius parameter

E: Height of the barrier

Q: Tunneling correction factor



- $Q = \frac{e^{\alpha}}{\beta - \alpha} (\beta e^{-\alpha} - \alpha e^{-\beta})$
- $\alpha = E/RT$
- $\beta = \frac{2a\pi^2(2mE)^{1/2}}{h}$



From  $\beta$  term: **very sensitive to the mass** of the tunneling particle  $\rightarrow \neq$  between H and D



**Huge impact on the kinetic**

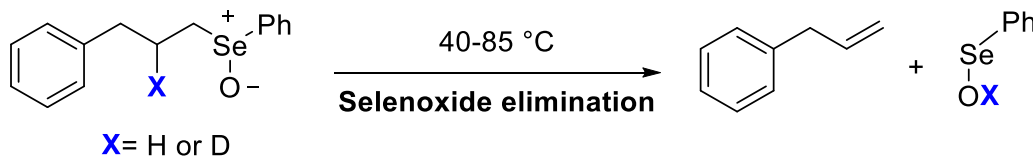
Lighter H atom can tunnel more readily than D

$\rightarrow$  KIE<sub>H/D</sub> value **higher** than theoretical maximum

# Types of Kinetic Isotope Effect (KIE)

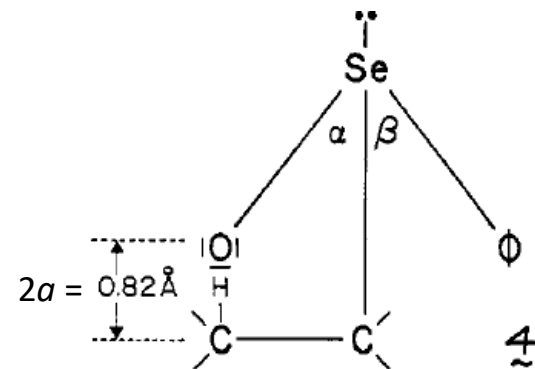
## Examples of tunneling effect:

- Selenoxide elimination (Kwart *et al.*, 1981)



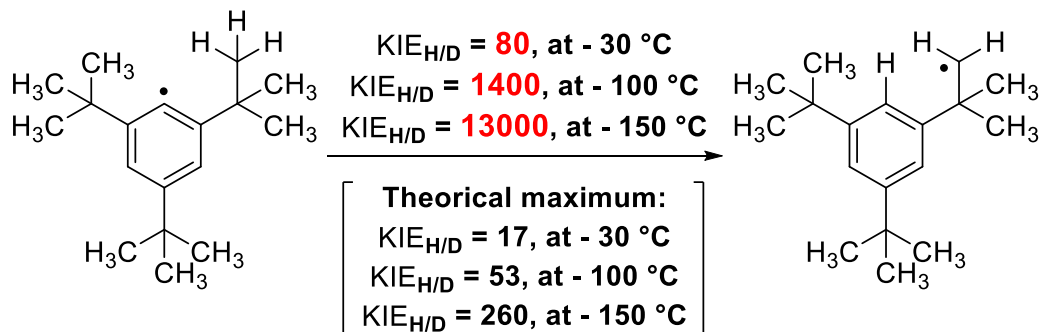
$$[\Delta E_a]^{H/D} = 2.52 \text{ kcal.mol}^{-1} \gg [\Delta E_0]^{H/D}$$

$$2a = 0.82 \text{ \AA} < \text{normal C-H bond (1.1 \AA)}$$



CYCLIC ARRANGEMENT LEADING  
TO TUNNELING H-TRANSFER  
IN SELENOXIDE THERMOLYSIS

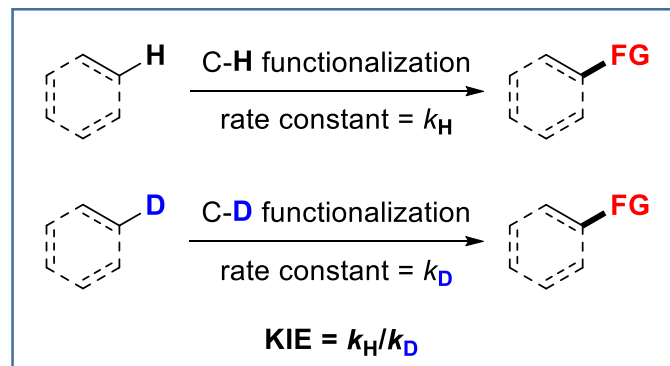
- Rearrangement of 2,4,6-tri-*tert*-butylphenyl radical (Ingold *et al.*, 1976)



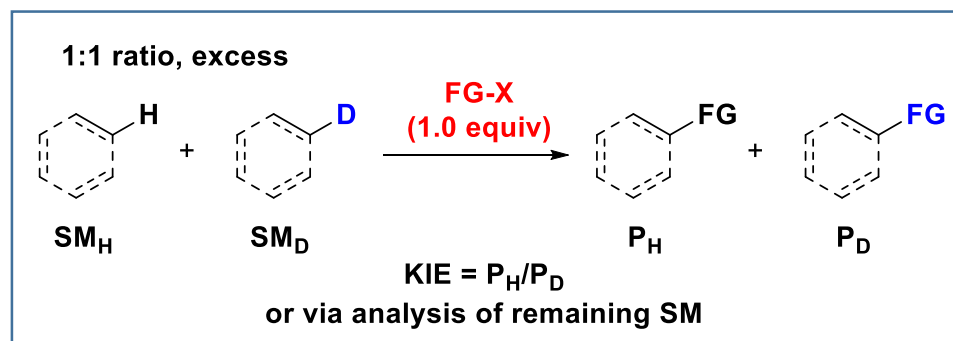
# Classical experiments

Three main experiments with deuterated substrates:

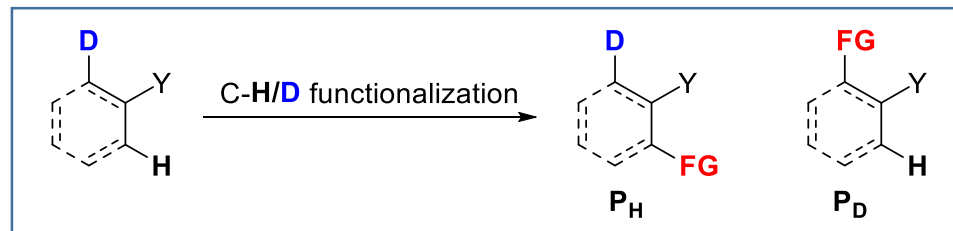
## 1. Parallel reactions



## 2. Intermolecular competition



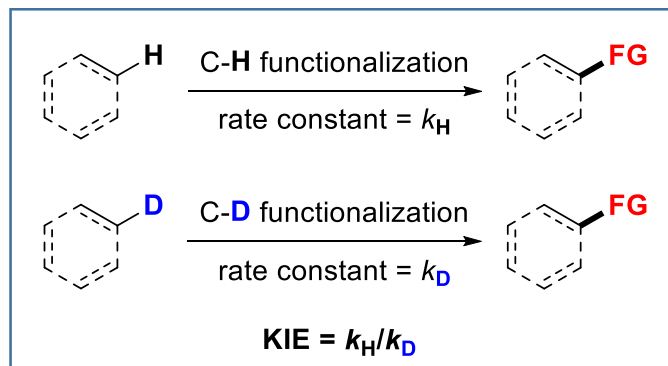
## 3. Intramolecular competition



# Classical experiments

Three main experiments with deuterated substrates:

## 1. Parallel reactions

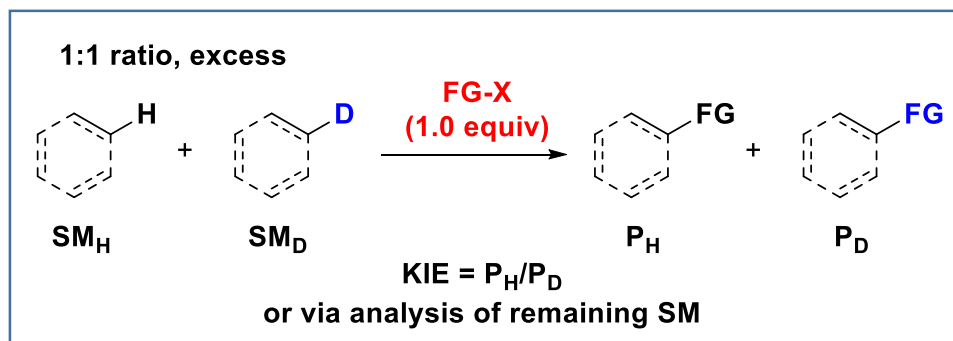


- Relative rates measured
- Affected by catalyst decomposition and induction periods
- **If strong primary KIE, C-H functionalization = rate-determining step** (= RDS)  
(turnover-limiting step for catalytic reaction)
- No KIE observed if C-H functionalization happen after RDS  
Possible small KIE if before RDS (in case of equilibrium isotope effect)

# Classical experiments

Three main experiments with deuterated substrates:

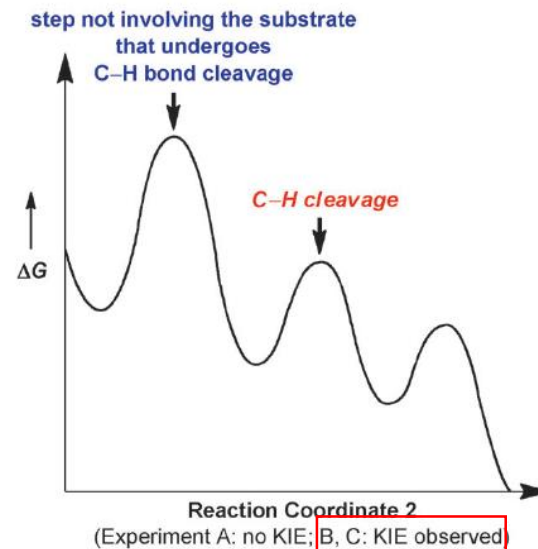
## 2. Intermolecular competition



- KIE measured from ratio  $\text{P}_H/\text{P}_D$  (i.e. 1:1 mixture cyclohexane:cyclohexane- $d_{12}$  used)
- Alternatively from consumed SM ratio
- **If no KIE, C-H bond cleavage not RDS**
- **If strong primary KIE, not necessary RDS !**  
(If RDS before and not involving the substrate)

Example: ligand dissociation, oxidative addition to another molecule, metal oxo formation, ...

→ C-H functionalization **product-determining step**

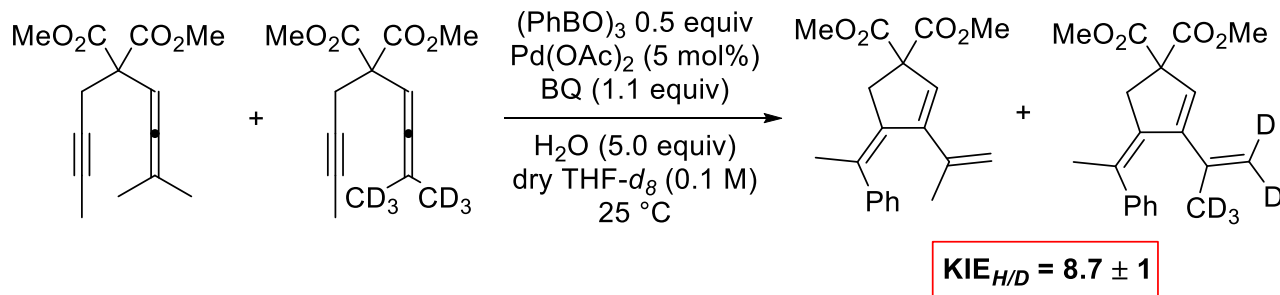




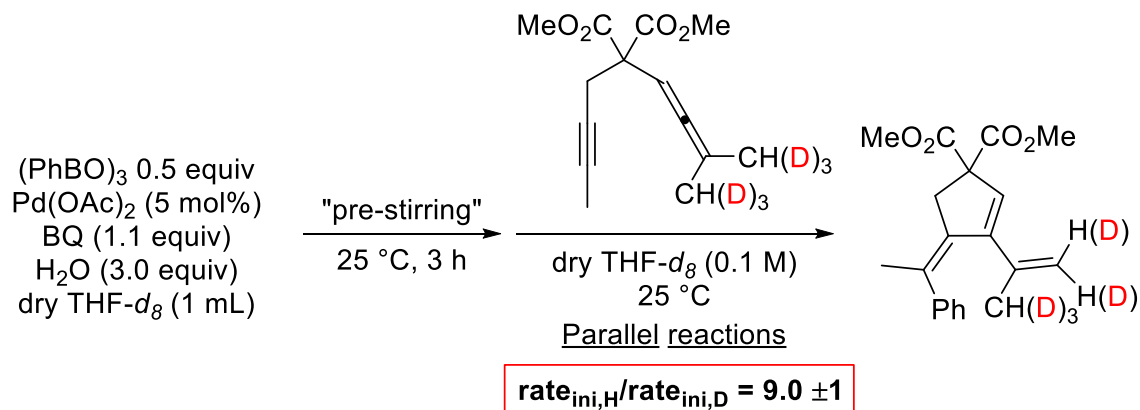
# Classical experiments

Example: Palladium-catalyzed oxidative arylating carbocyclization of allenynes (Bäckvall et al., 2018)

- **Intermolecular competition**



- **Parallel reaction → induction period: required pre-stirring**



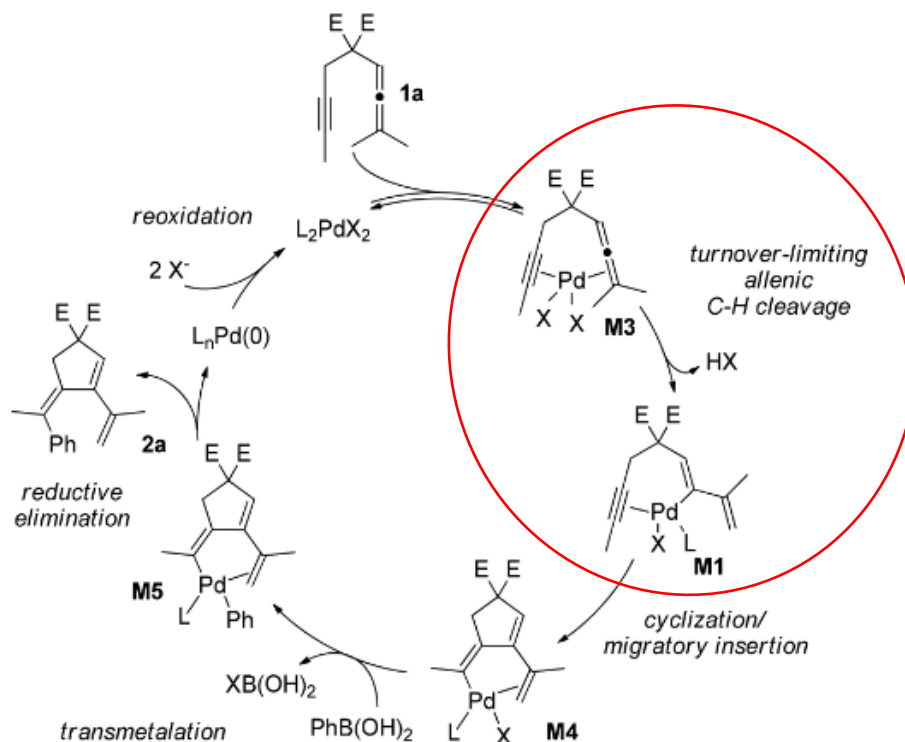
# Classical experiments

Example: Palladium-catalyzed oxidative arylating carbocyclization of allenynes (Bäckvall et al., 2018)

**KIE (competition experiment) = 8.7  $\approx$  KIE (parallel experiment) = 9.0**

**→ Strong KIE: Allenic C-H cleavage turnover-limiting step**

Proposed mechanism:



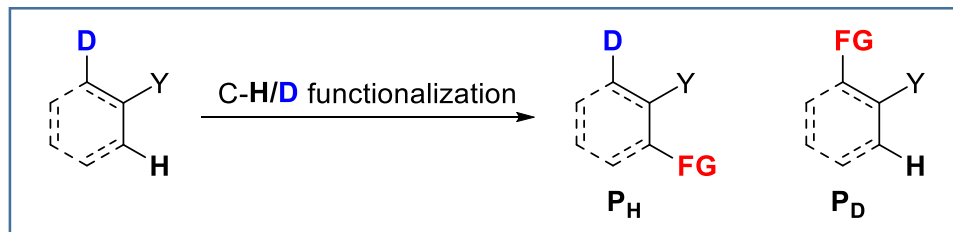
L = neutral ligand (e.g. BQ, HQ, THF, H<sub>2</sub>O, etc.)

X = anionic ligand (e.g. AcO<sup>-</sup>, HOC<sub>6</sub>H<sub>4</sub>O<sup>-</sup>, etc.)

# Classical experiments

Three main experiments with deuterated substrates:

## 3. Intramolecular competition

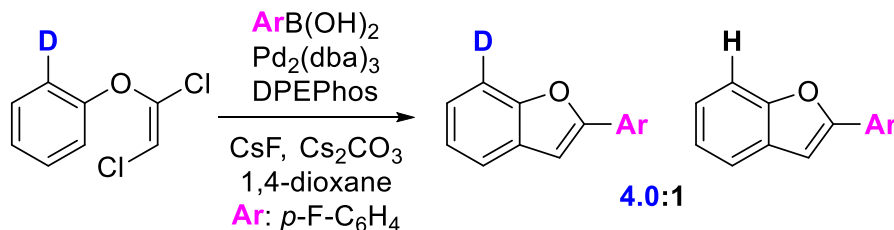


- KIE measured from ratio  $P_H/P_D$
- Often Y = directing group
- **If no KIE, C-H bond cleavage not RDS**
- **If strong primary KIE, not necessary RDS !**  
(If RDS before or equilibrium)

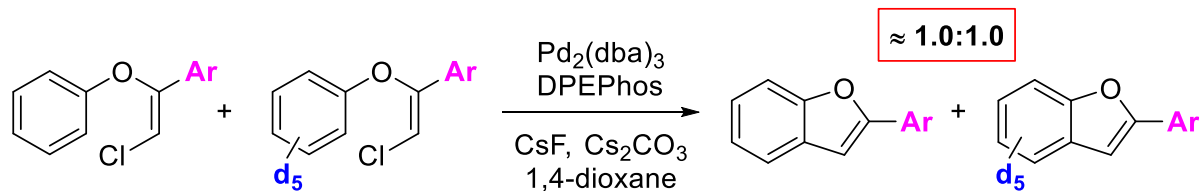
# Classical experiments

Example: Pd-catalyzed one-pot Suzuki-Miyaura coupling/C-H direct arylation (Hultin *et al.*, 2010)

- Intramolecular competition: Strong KIE



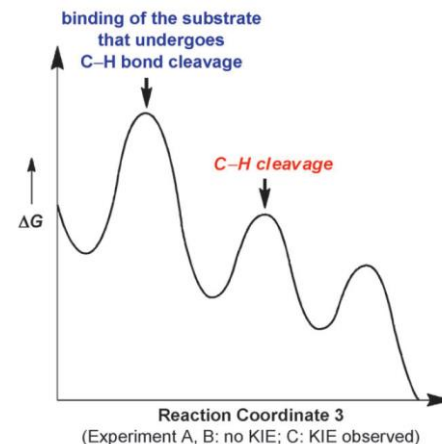
- Intermolecular competition: no KIE



→ C-H functionalization not turnover-limiting step



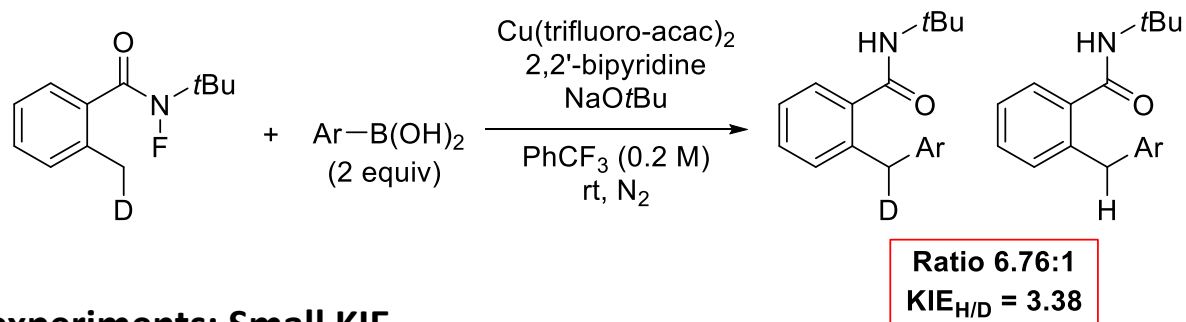
Turnover-limiting step: **Oxidative addition to C-Cl bond**



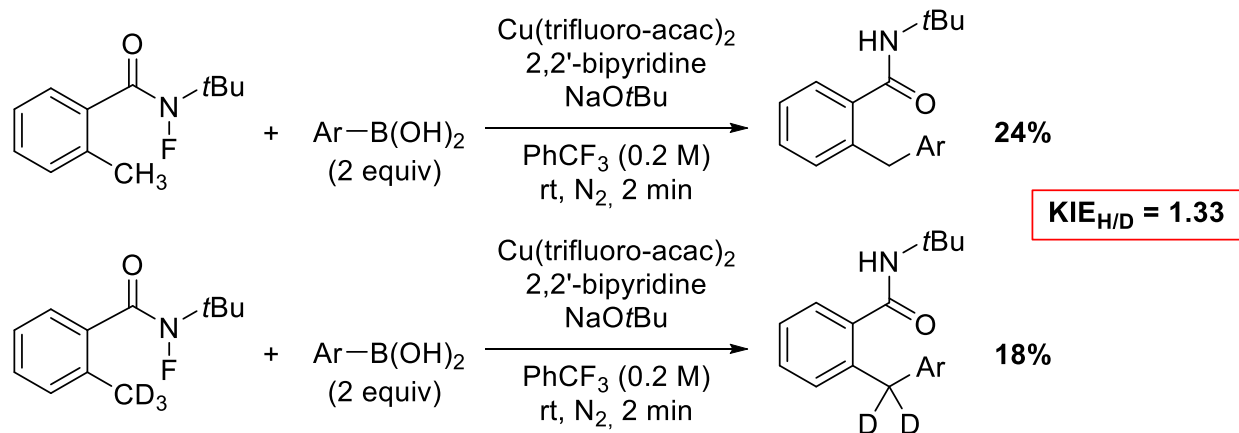
# Classical experiments

Example: Cu-catalyzed arylation of remote C(sp<sup>3</sup>)-H bonds in carboxamides and sulfonamides  
(Our group – 2018)

- Intramolecular competition: Strong KIE<sub>H/D</sub>**



- Parallel experiments: Small KIE<sub>H/D</sub>**



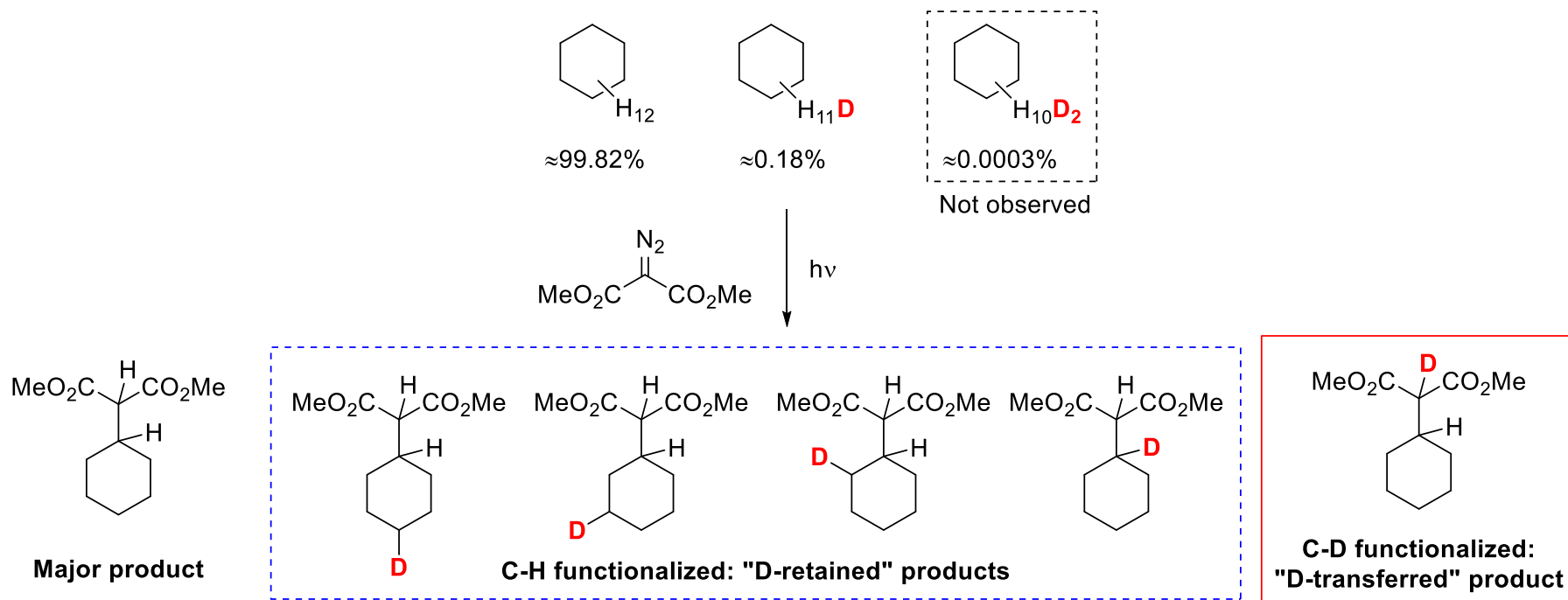
**Only minimal contributions of the 1,5-HAT in the overall reaction rate**

# KIE measured at natural-abundance

Deuterium kinetic isotope effects by natural-abundance deuterium NMR spectroscopy

(Pascal *et al.*, 1984)

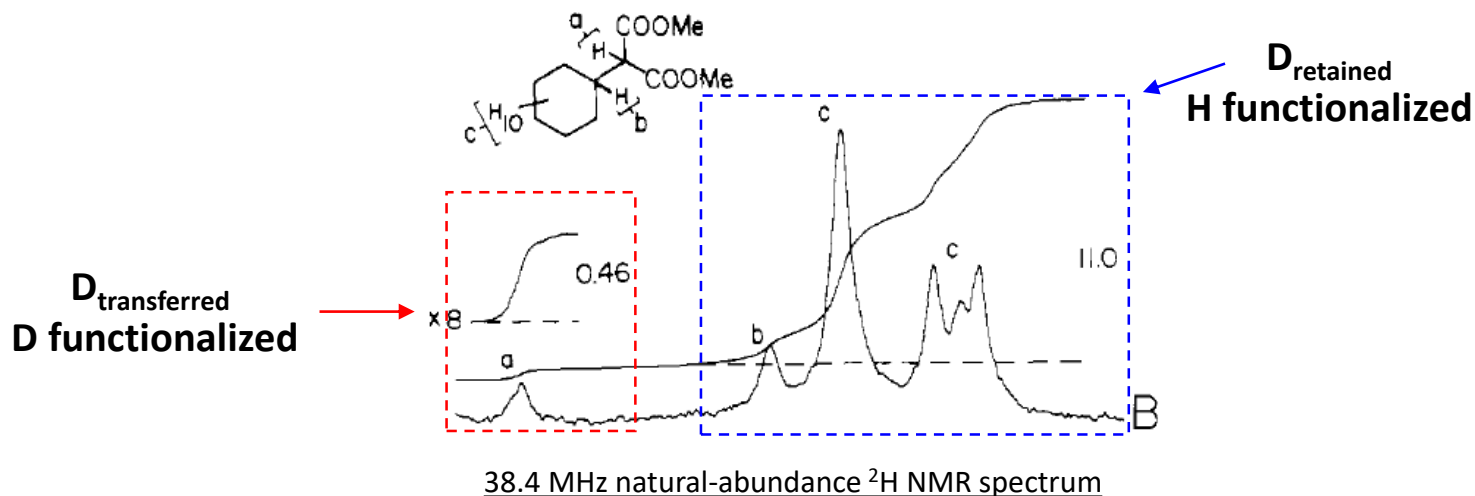
$^2\text{H}$  natural abundance: 0.01-0.02%



**Formula  $\text{KIE}_{\text{H/D}}$ :**  $k_{\text{H}}/k_{\text{D}} = (D_{\text{retained}}/D_{\text{transferred}})[1/(n-1)]$

$n$ : number of chemically equivalent sites in the reactant

# KIE measured at natural-abundance



$$k_{\text{H}}/k_{\text{D}} = (D_{\text{retained}}/D_{\text{transferred}})[1/(n-1)] = (11.0/0.46)[1/(12-1)] = \mathbf{2.2}$$

Control experiment: Photolysis in presence of 1:1 cyclohexane:cyclohexane- $d_{12}$

$k_{\text{H}}/k_{\text{D}} = 2.2$  (determined by GC-MS)  $\rightarrow$  Agreement of the methods

Interpretation: **Transfer of C-H/D bond  $\rightarrow$  During the rate-determining step**

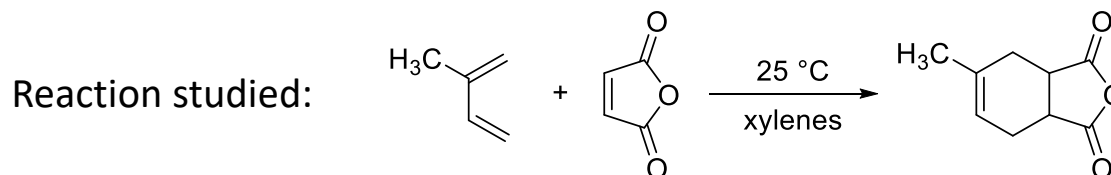
Limitations: **Low resolution/lower sensitivity**

$\rightarrow$  Require good amount of product and good separation of the peaks

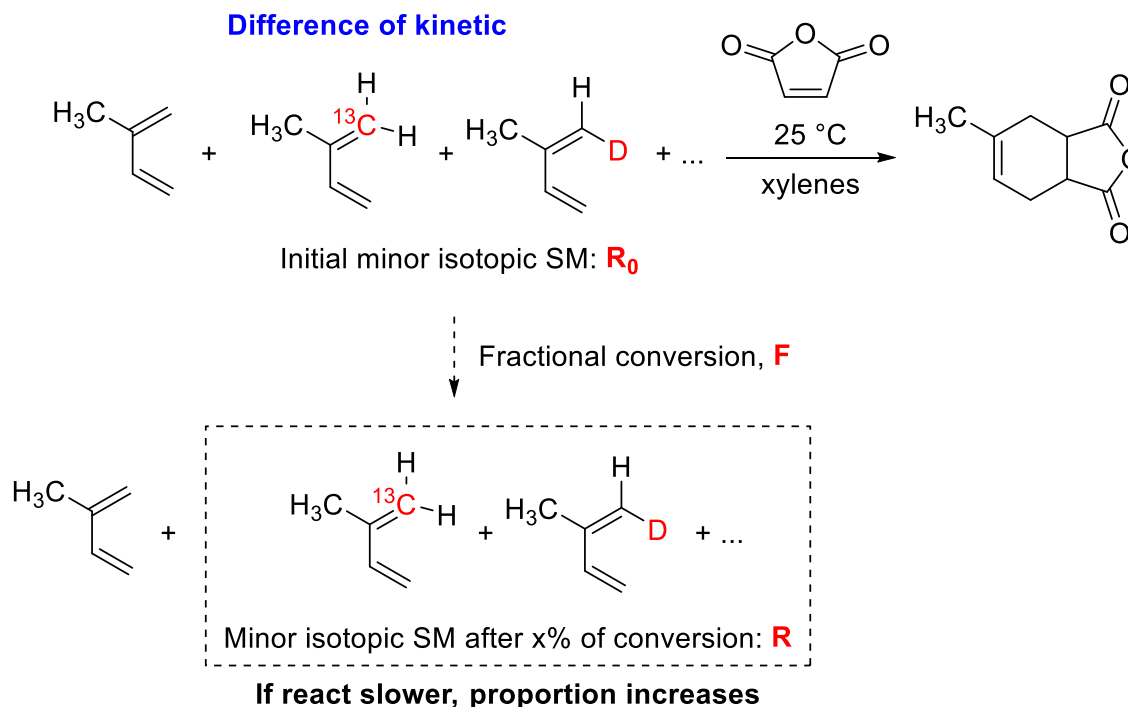
$\rightarrow$  In some cases, uncertainty on the exact value of KIE

# KIE measured at natural-abundance

High-precision determination of small  $^2\text{H}$  and  $^{13}\text{C}$  KIE at natural abundance (Singleton and Thomas, 1995)



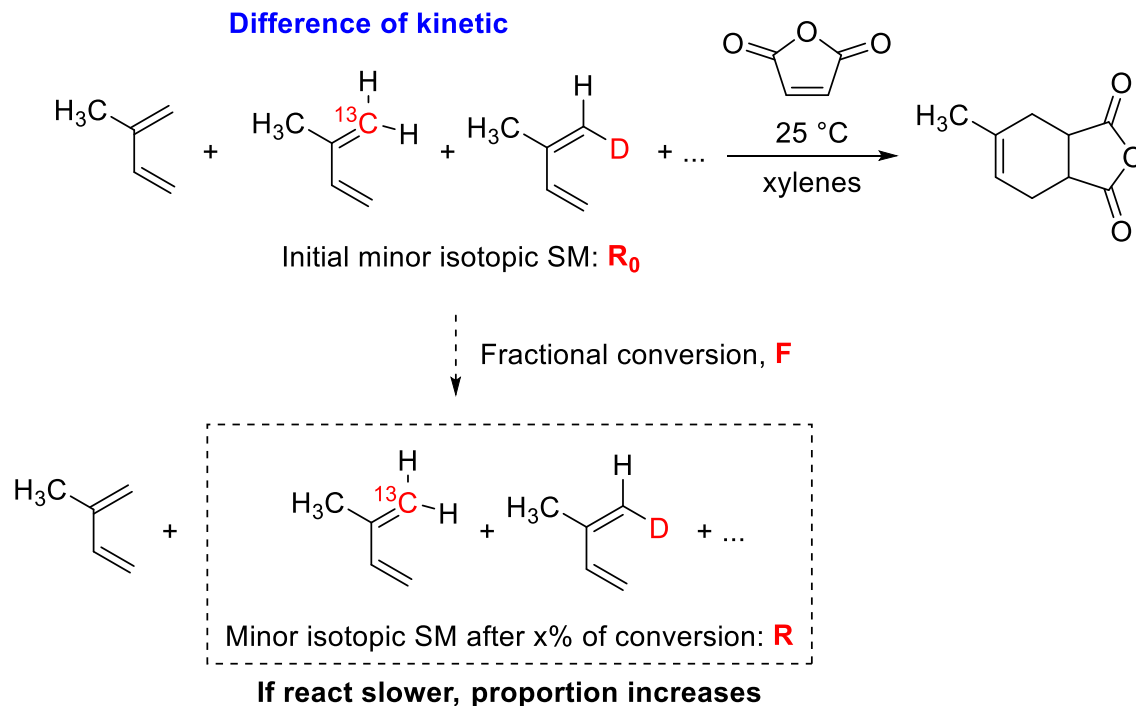
Principle: Fractional enrichment of the isotopically slower-reacting starting materials during the reaction.





# KIE measured at natural-abundance

Principle: Fractional enrichment of the isotopically slower-reacting starting materials during the reaction.



$$KIE_{\text{calcd}} = \ln(1-F)/\ln[(1-F)R/R_0]$$

F: “fractional” conversion of reactant

$R/R_0$ : proportion of minor isotopic component in recovered starting material compared to the original starting material

# KIE measured at natural-abundance

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In practice: The reaction is stopped before the end, at a conversion (F) > 70% (here, 98.9%).

$$\text{KIE}_{\text{calcd}} = \ln(1-F)/\ln[(1-F)R/R_0]$$

Uncertainties in calculated small KIE determined by NMR integrations (relatively low precision) dominated by  $\Delta\text{KIE}_R$ :

$$\Delta\text{KIE}_R = \frac{\delta\text{KIE}}{\delta(R/R_0)} \Delta(R/R_0) = \frac{-\ln(1-F)}{(R/R_0)\ln^2[(1-F)R/R_0]} \Delta(R/R_0)$$

→ More F increases, smaller becomes  $\Delta\text{KIE}_R$

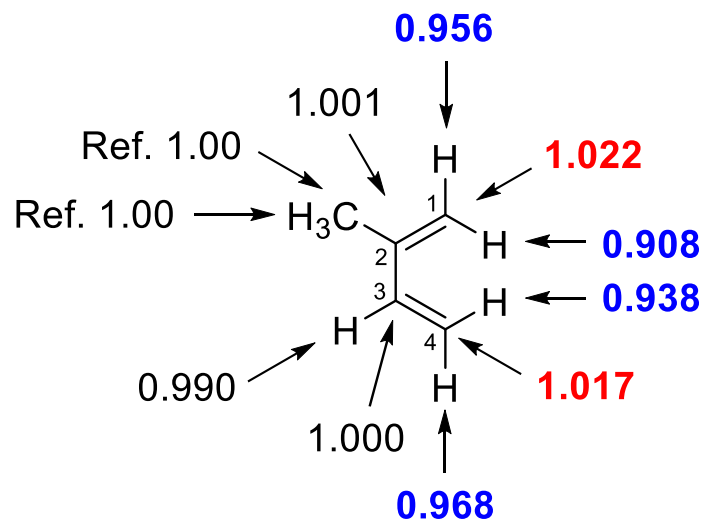
Consequence: Higher is the conversion, higher is the **precision for small KIE determinations**

NB: Possible to analyze the product when F < 20%.

(Other parameter to take in account in cases of large KIE or if (R/R<sub>0</sub>) can be very precisely determined:  $\Delta\text{KIE}_F$ )

# KIE measured at natural-abundance

Classical  $\text{KIE}_{12\text{C}/13\text{C}}$  values:  $0.99 < \text{KIE} < 1.04$



→ CH<sub>3</sub> used as internal reference.

→ KIE for C2, C3, H3: no/very small KIE (as expected for non-reacting atoms).

→ Important **KIE for C1 and C4** (Slower reaction with C1 or C4 = <sup>13</sup>C).

→ Important **inverse KIE for H1 and H4** (faster reaction with H1 and H4 = D).

## Interpretation:

KIE at C1 and C4 consistent with the **concerted mechanism** proposed

Inverse KIE for H1 and H4 results from the **sp<sup>2</sup> → sp<sup>3</sup> rehybridization** in the TS

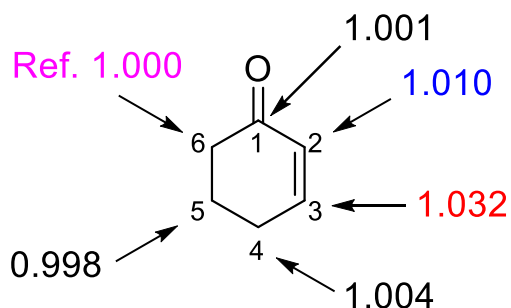
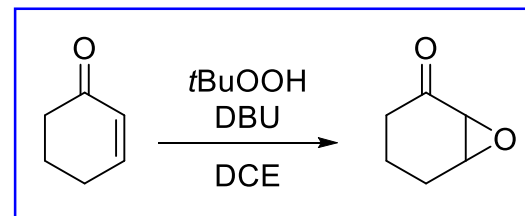
Difference in KIE for H1s and H4s suggests some **asynchronicity** in bond formation

# KIE measured at natural-abundance

Example: Mechanism of epoxidation of cyclohexenone with *tert*-butyl hydroperoxide (Singleton *et al.*, 2007)

→ Investigations to determine rate-determining step

→ Natural-abundance  $\text{KIE}_{12\text{C}/13\text{C}}$  determined



	Predicted KIEs				
axial addition (10)	1.004	1.008	1.027	0.997	0.999
equatorial addition (13)	1.004	1.007	1.031	1.001	1.001
axial ring-closure (12)	1.006	1.012	0.997	0.996	0.999
equatorial ring-closure (15)	1.005	1.012	1.000	0.999	1.001

**Strong primary  $\text{KIE}_{12\text{C}/13\text{C}}$  at C3** and moderate one at C2 → Match with predicted KIEs

➡ **1,4-addition of  $\text{tBuOO}^-$  rate-limiting step**

# KIE measured at natural-abundance

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## Advantages:

- **No synthesis of deuterated substrate necessary**
- Measurements of detailed **small KIE can be quickly obtained.**
- Low influence of impurities in the analyzed compounds (if not overlapping in NMR)
- Access to important information on reacting atoms.

## Limitations:

- Require the reaction to be **scalable** enough to recover remaining starting materials (few %) and that it could be isolated from a large amount of products.

In Singleton and Thomas' case, they distilled off the remaining isoprene from the reaction mixture (at 98,9% of conversion, starting from 1.0 mol of isoprene).

- The reaction should be **irreversible**.
- The reaction mechanism must not change during the course of the reaction

# KIE measured at natural-abundance

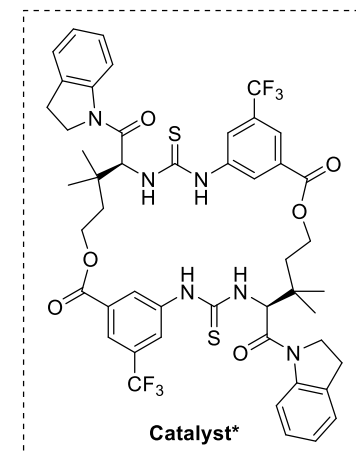
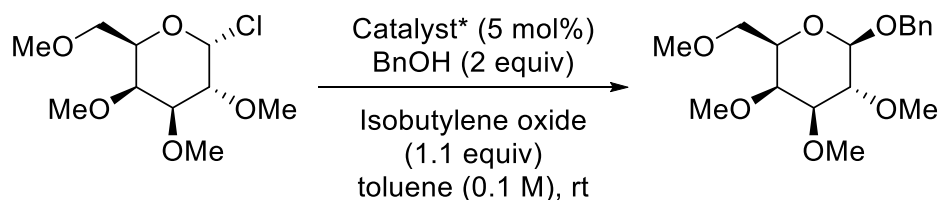
## Use of polarization transfer NMR for $^{13}\text{C}$ KIE measurements at natural abundance

(Jacobsen *et al.*, 2017)

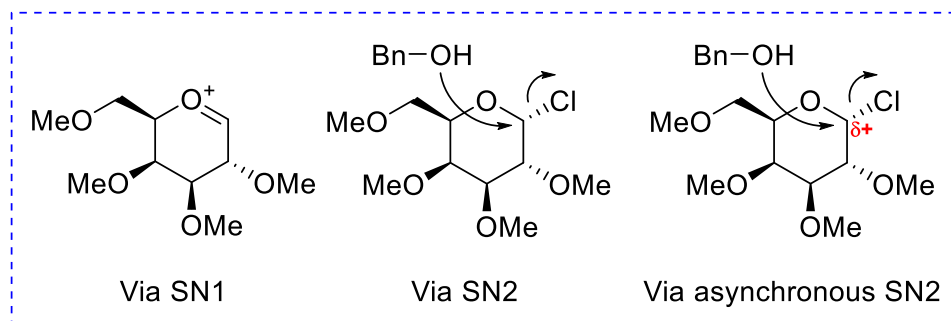
Principle: DEPT (distortionless enhancement by polarization transfer)

→ Transfer the larger gyromagnetic ratio of  $^1\text{H}$  to  $^{13}\text{C}$  to increase the sensitivity (x3) or reduce the time of experiment acquisition (x9)

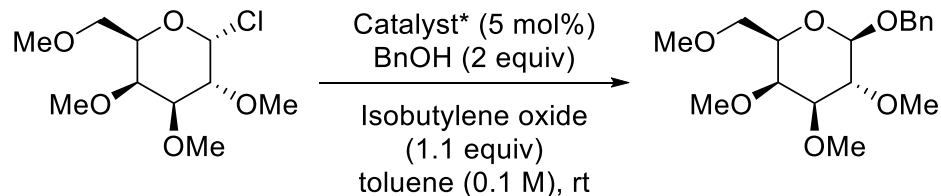
Example: Investigation of this thiourea-catalyzed glycosylation



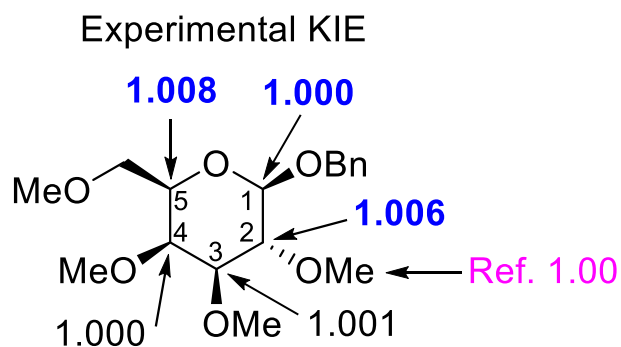
**Mechanism ?**



# KIE measured at natural-abundance



## Measurement of $^{13}\text{C}$ KIE and comparison to predicted value



$\text{S}_{\text{N}}^2$  classical  $^{13}\text{C}$  KIE range = 1.03-1.09

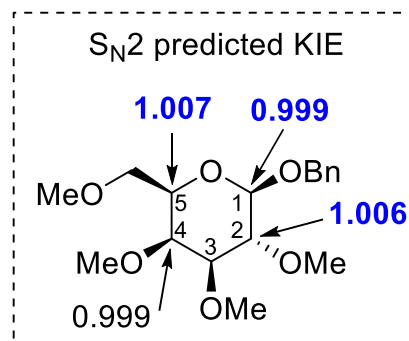
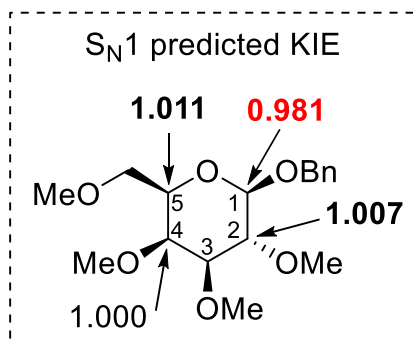
$\text{S}_{\text{N}}^1$  classical EIE range = 0.98-1.01

Asynchronous  $\text{S}_{\text{N}}^2$  classical  $^{13}\text{C}$  KIE range = 0.99-1.02

Small/normal KIEs observed for C1, C2 and C5

→ Low for a synchronous  $\text{S}_{\text{N}}^2$  process

→ Indicating significant **oxocarbenium character** in the TS



# KIE measured at natural-abundance

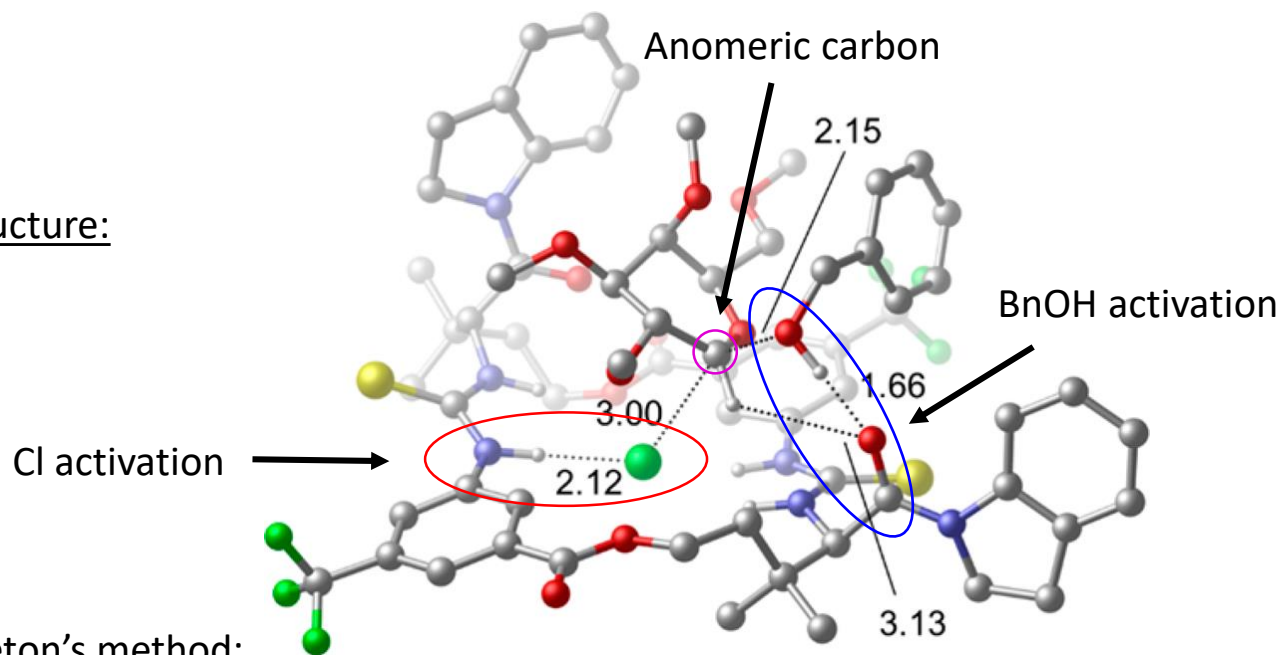
## Conclusion:

- Significant oxocarbenium character in the transition state
- Suggest **asynchronous** reaction mechanism with a **large degree of charge separation**

**Could not conclude on the exact mechanism**

- Cooperative mechanism via dual activation of Nu and E

## DFT calculated transition structure:



## Difference compared to Singleton's method:

- Only  $^{13}\text{C}$ -H accessible (DEPT)
- **Increase in sensitivity** (Lower amount required or shorter acquisition time).



# Conclusion and outlook

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- **Very powerful information:**
    - On the rate-determining step (primary KIE)
    - On the mechanism (comparison of mechanisms, solvent effect, ...)
  - Particularly used for **C-H functionalization** (Large values for primary KIE)
  - Routinely used nowadays
- 8195 references on Scifinder containing the words “kinetic isotope effect” in the abstract

**However, careful choice of the experiments necessary → easy to draw wrong conclusions**

- Most of the time → Required synthesis of **deuterated substrate**

Alternatively, **KIE measured at natural-abundance**

→ Often require scalable reaction: **low sensitivity** or **long acquisition time experiments**



**Development of more sensitive methods**

# Conclusion and outlook

- Development of more sensitive methods

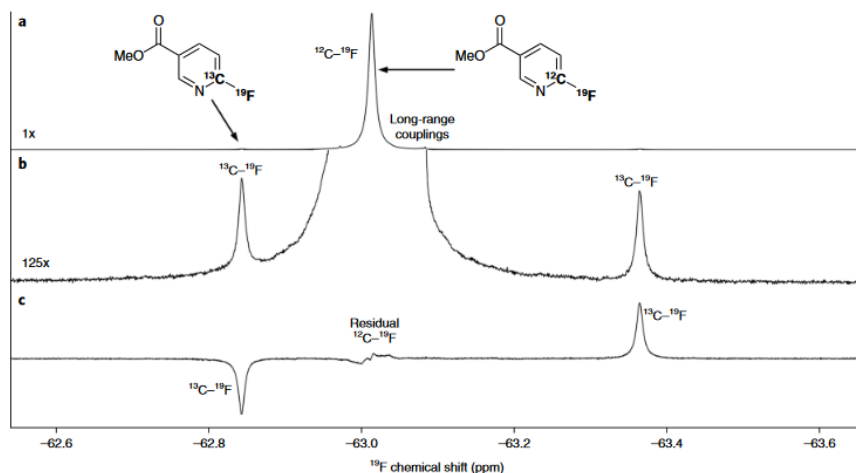
Example: Use of MQF NMR for Concerted  $S_NAr$  (Jacobsen et al., **2018**)

Use of Multiple-quantum filtered spectra (MQF) to obtain  $KIE_{12C/13C}$

MQF =  $1^9F$  NMR {1 H decoupled} NMR where parent  $^{12}C$ - $^{19}F$  peak removed

→ Only  $^{13}C$ - $^{19}F$  satellite integrals remaining: accurate integration

→ **Hugh gain in sensitivity: 13.6 times** (per F) greater than  $^{13}C$  NMR signal



**Fig. 2 | Assessing  $^{13}C$  isotopic fractionation by suppressing NMR signals from fluorine atoms bound to  $^{12}C$ .** **a**, Standard  $^{19}F$ { $^1H$ } spectrum showing the parent  $^{12}C$ - $^{19}F$  peak flanked by two  $^{13}C$ - $^{19}F$  satellites. **b**, The standard spectrum in **a**, enlarged 125x. Accurate satellite integrals cannot be obtained directly due to overlap with the parent peak. **c**, MQF  $^{19}F$ { $^1H$ } spectrum. Suppression of the parent peak allows accurate integration of the satellites.

**Table 1 | Comparison of KIE measurements**

Method	Time (h)	KIE (s.e.)
1 Singleton (300 mg) <sup>a</sup>	9.9	1.058(6), 1.060(6)
2 MQF (50 mg) <sup>b</sup>	4.6	1.057(5), 1.065(6)
3 MQF (50 mg)	5.2	1.057(3), 1.062(4)
4 MQF (50 mg) <sup>c</sup>	5.2	1.059(4), 1.060(4)
5 MQF (10 mg)	9.9	1.061(6)
6 MQF (50 mg) <sup>d</sup>	0.7	1.055(10)
Consensus (s.d.):		<b>1.059(3)</b>

Samples are pure unless otherwise noted. Acquisition times are given for each pair of partial and full conversion samples. Pairs of KIEs refer to independent chemical replicates. Error bars (in parentheses) refer to standard errors (s.e.) of the mean (t distribution) and reflect technical variation due to errors in the measurement of conversion and satellite area. KIEs are referenced to  $^{12}C$ - $^{19}F$  unless otherwise noted. s.d., standard deviation. <sup>a</sup>KIEs are referenced to  $^{13}C$ - $^{19}F$ . <sup>c</sup>Unpurified sample. <sup>d</sup>Shigemi tube used.

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**Thank you for your attention**