Synthetic Strategies for ^{14}C Labelling of Drug Molecules

Dr Sean L Kitson

sean.kitson@almacgroup.com

Objective

- This lecture will focus on a brief introduction to ‘what is’ carbon-14
- Then leading onto some synthetic strategies towards labelling potential drug molecules with carbon-14

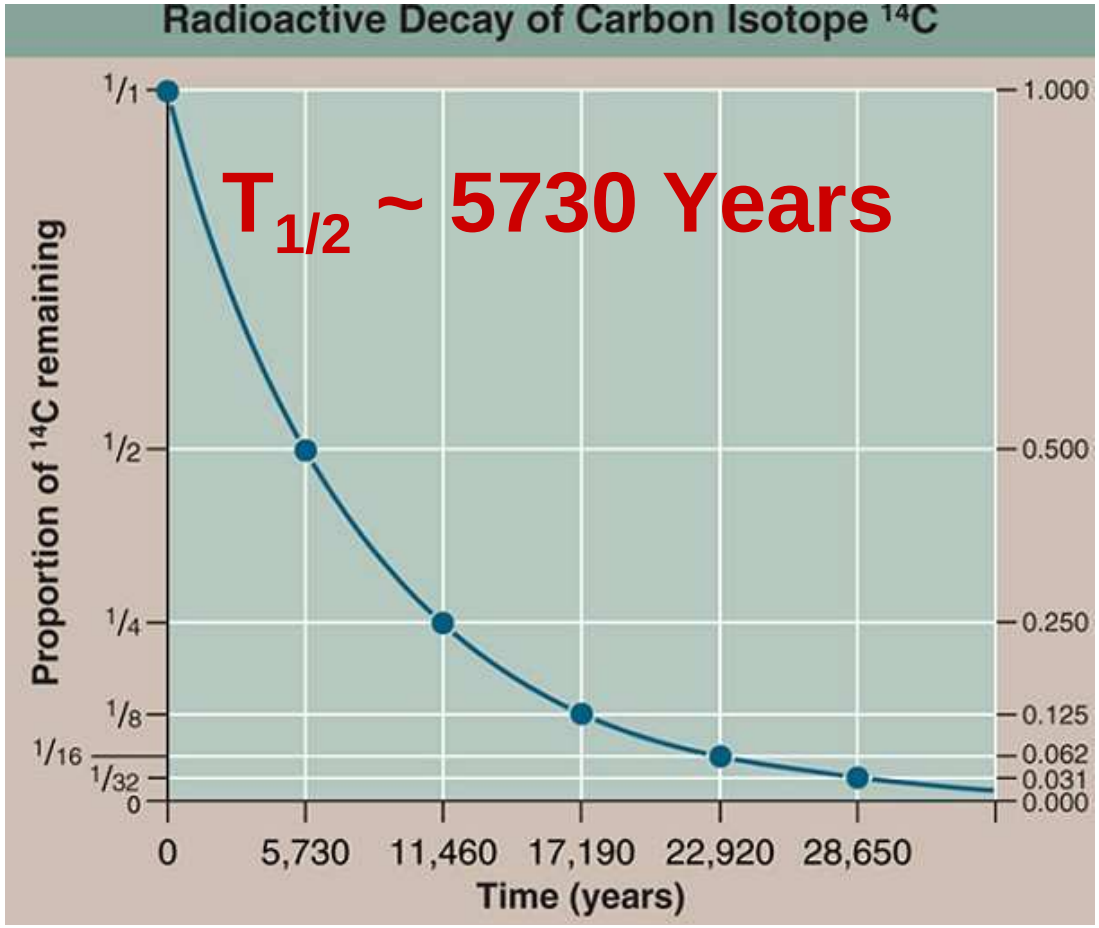
Agenda

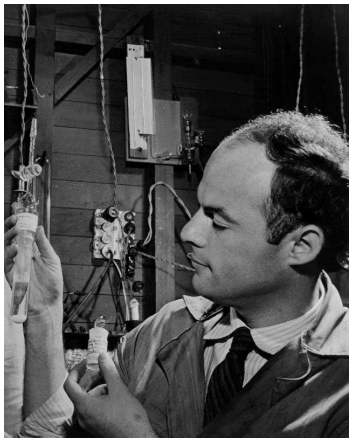
- Introduction to ^{14}C
- ^{14}C Synthetic Strategies:
  - [^{14}C]XEN-D0401
  - [^{14}C]Apomorphine
  - [^{14}C]Combretastin-A1
  - [5-^{14}C]Hex-5-ynoic acid
  - [^{14}C]ZT-1
- Conclusion

Introduction to ^{14}C

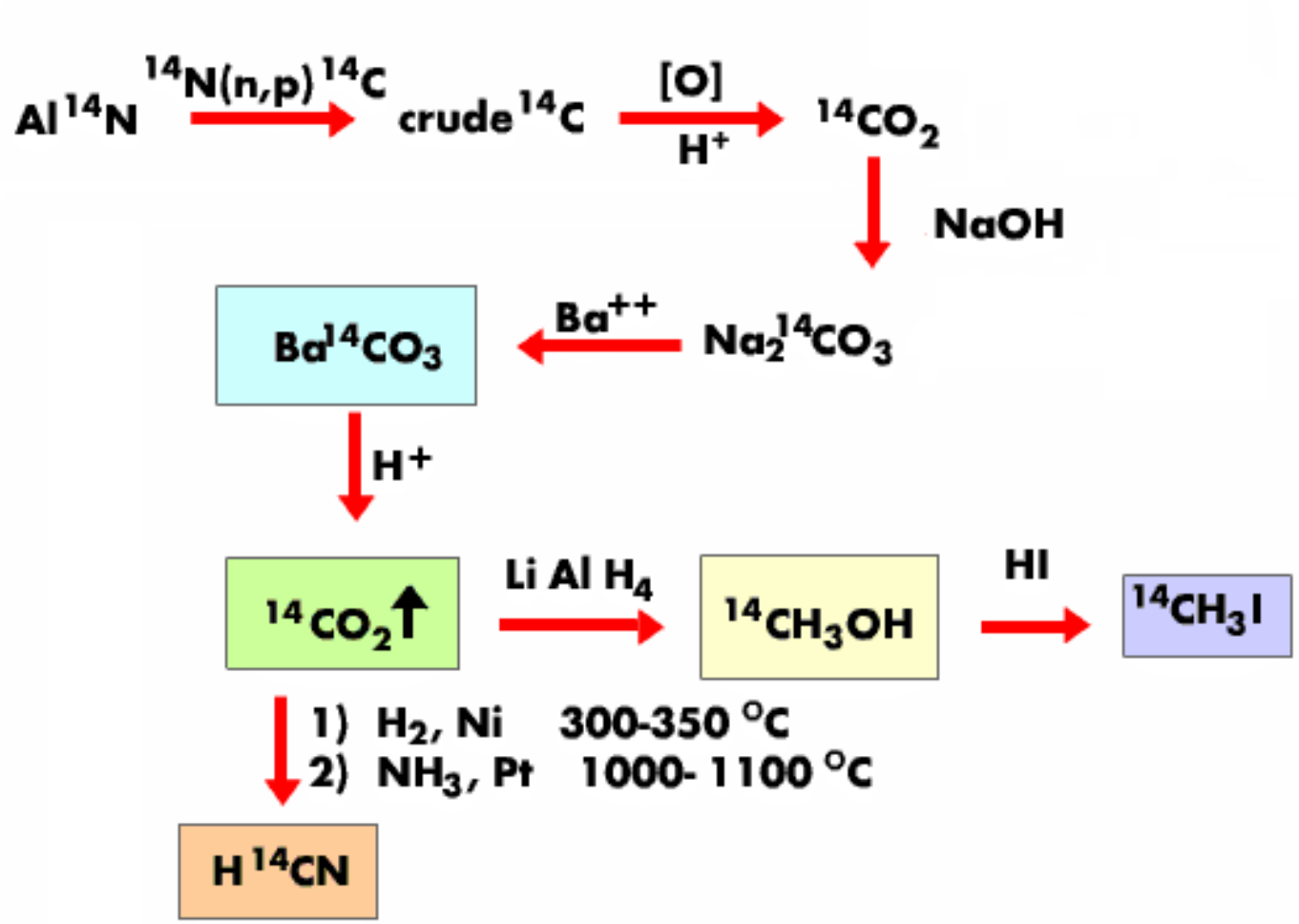
Discovery of ^{14}C

Martin Kamen & Sam Ruben (27-FEB-1940)

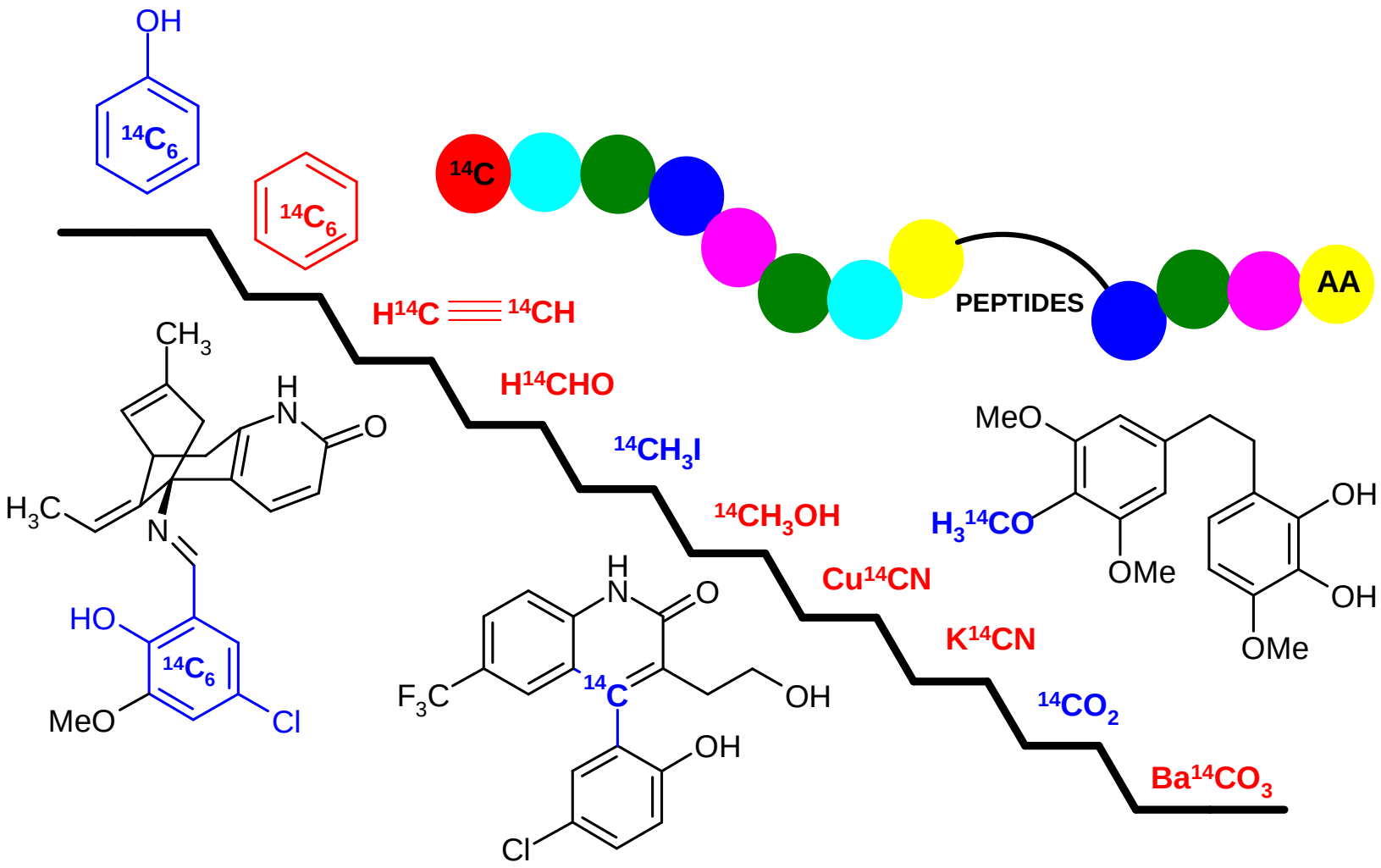




^{14}C Starting Materials

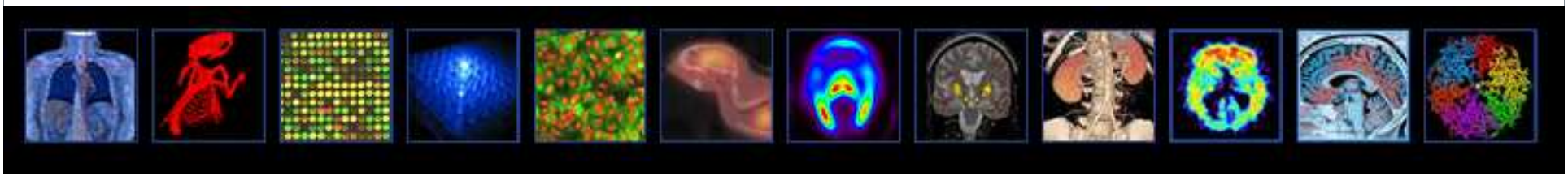


Barium ^{14}C carbonate staircase



^{14}C Radiotracer

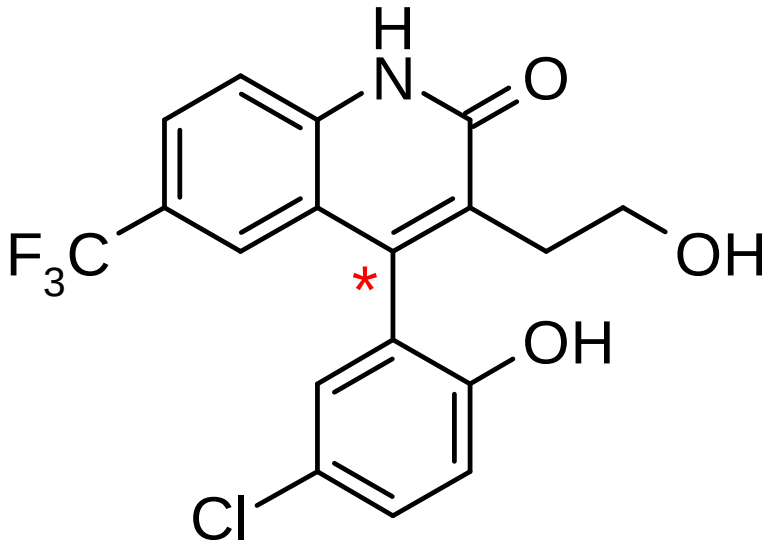
- In pharmaceutical research ^{14}C is used as a tracer to ensure that potential drugs are metabolized without forming harmful by-products – **ADMET Studies**
- The ^{14}C label should ideally form part of the compounds molecular skeleton



^{14}C Synthetic Strategies

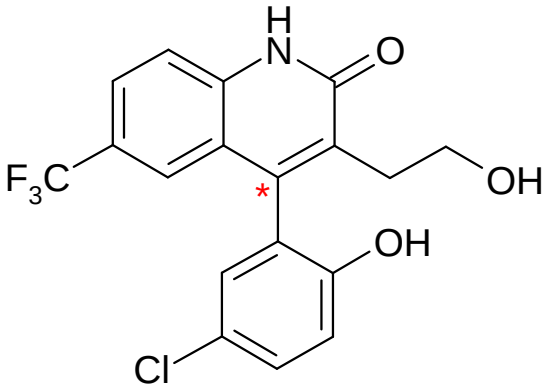
Target: [^{14}C]XEN-D0401

- XEN-D0401 is a novel and selective small molecule activator of the large-conductance calcium-activated potassium channel (known as the BK channel)
- Currently in Phase 1 development for the treatment of overactive bladder (OAB)

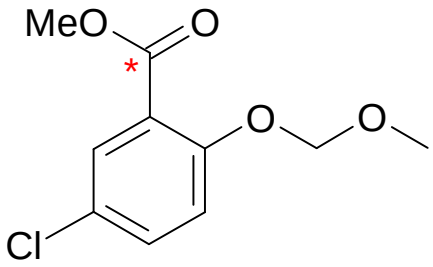


*** = ^{14}C Label**

^{14}C Starting Material



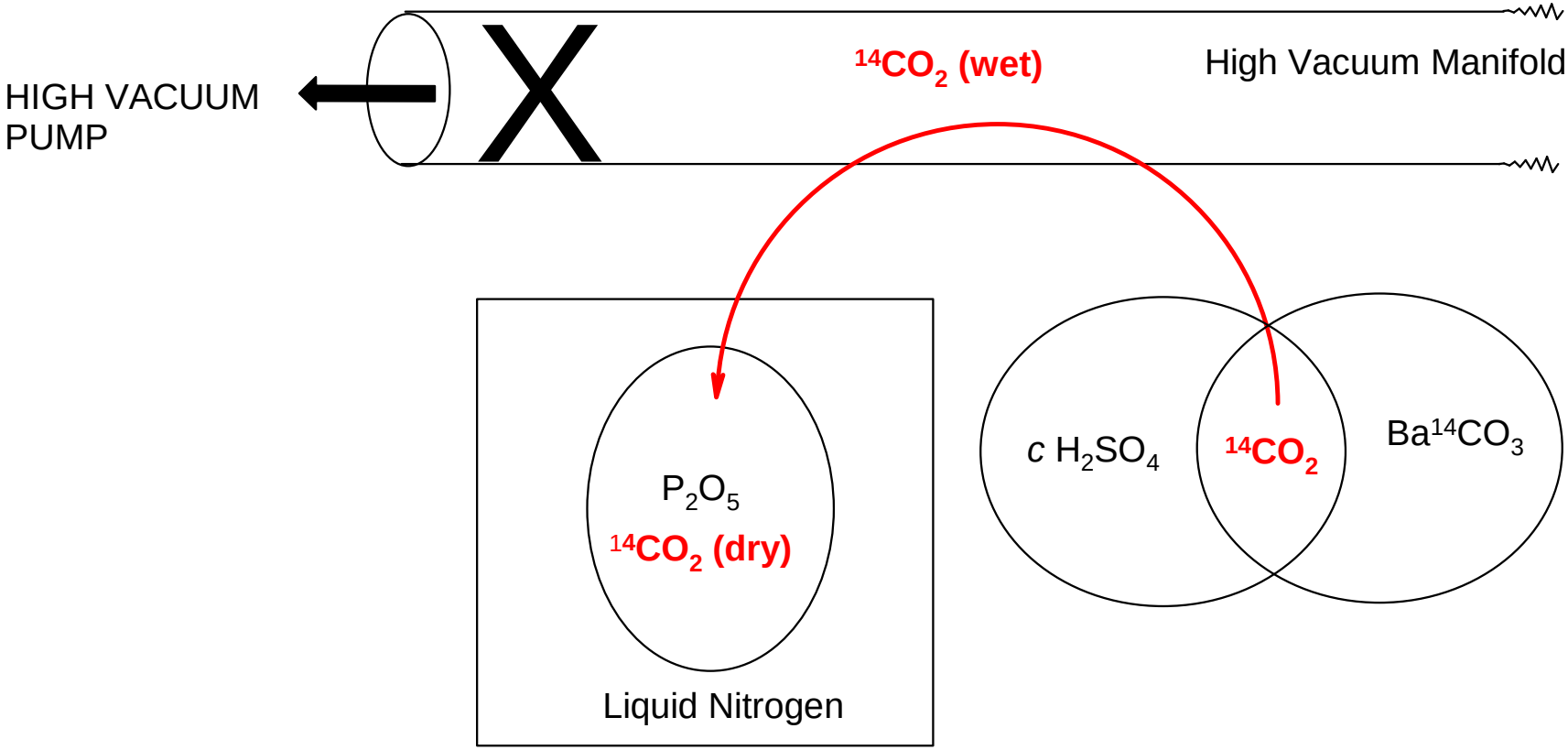
[^{14}C]XEN-D0401



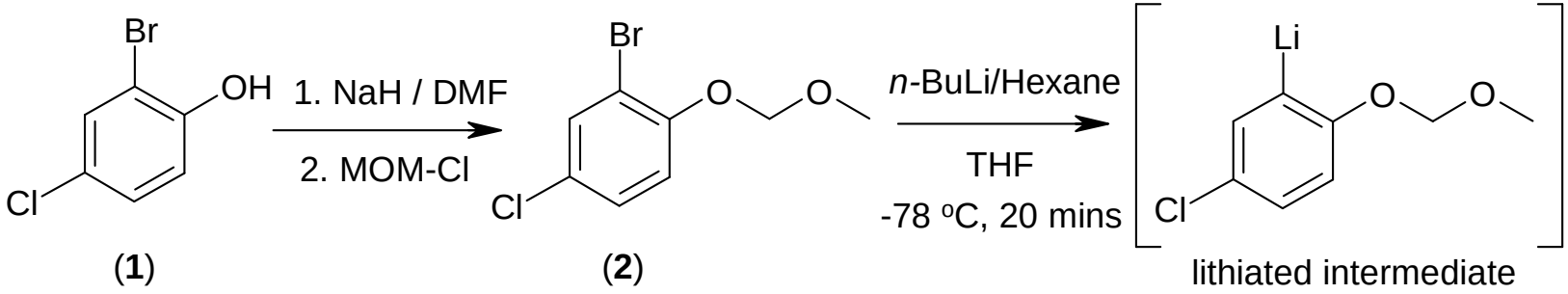
[^{14}C]-4

$^{14}CO_2$

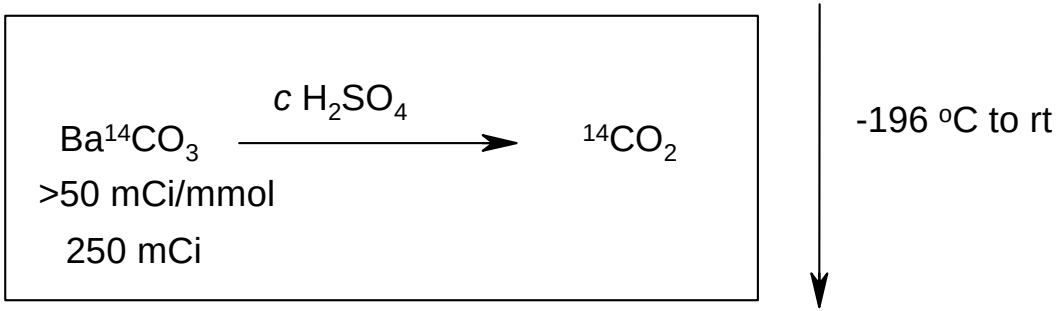
Generation of ‘Dry’ $^{14}CO_2$



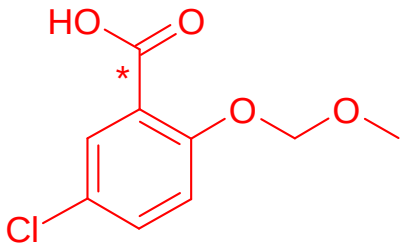
Carboxylation



(**1**) (**2**) lithiated intermediate

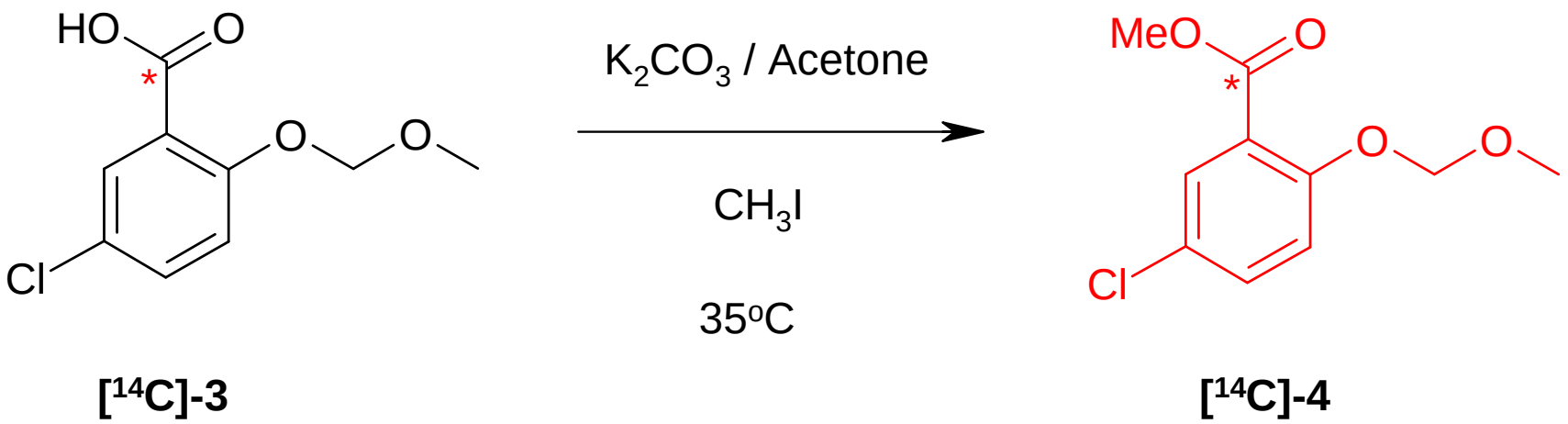


Radiochemical yield = 76%



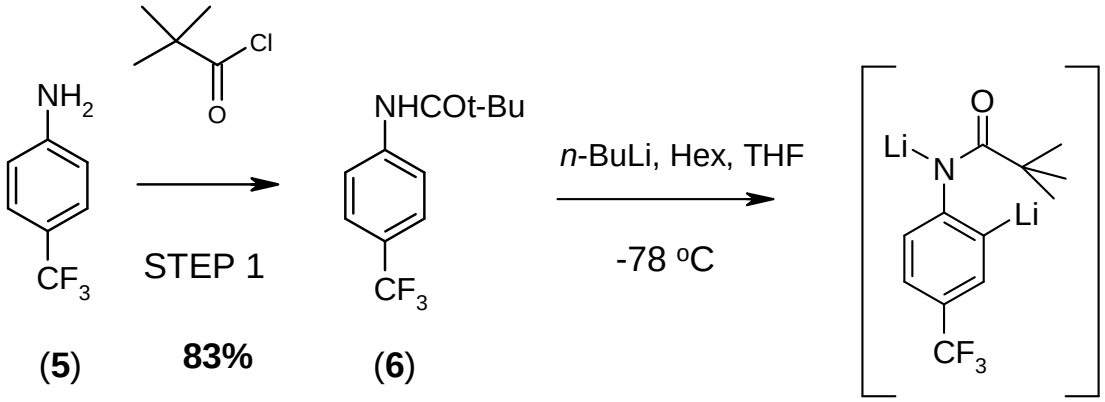
[^{14}C]-3

O-Methylation

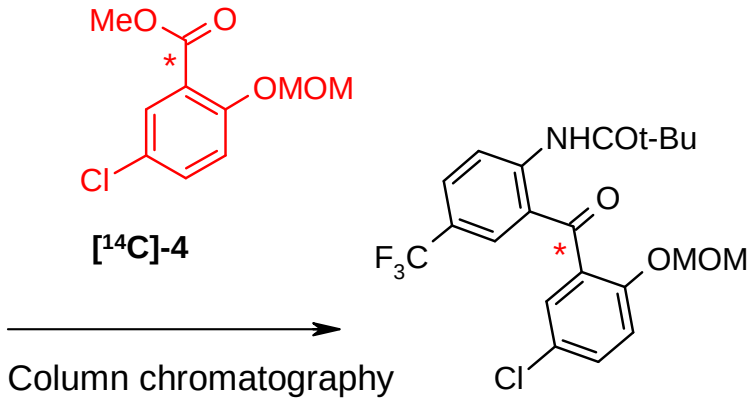


[^{14}C]-3

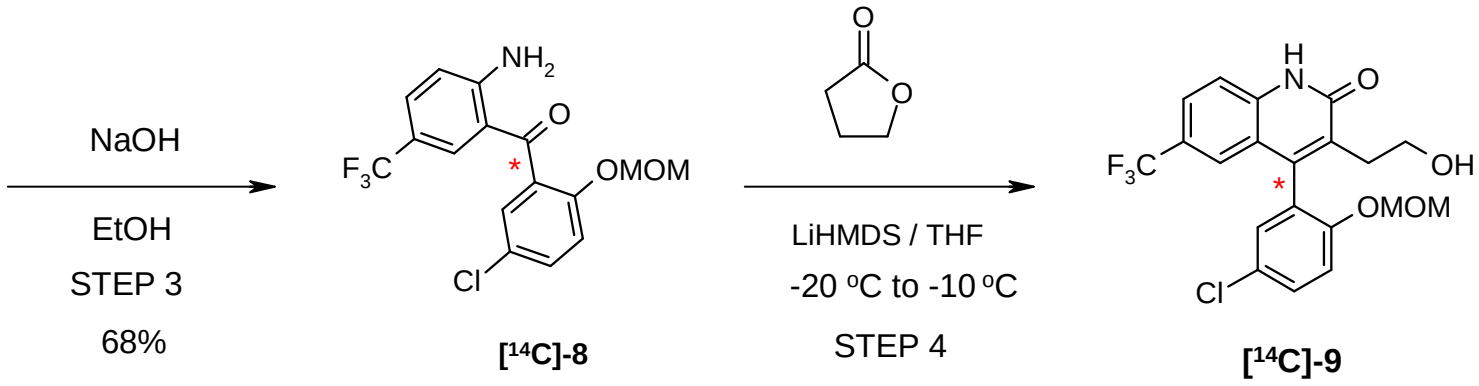
[^{14}C]-4

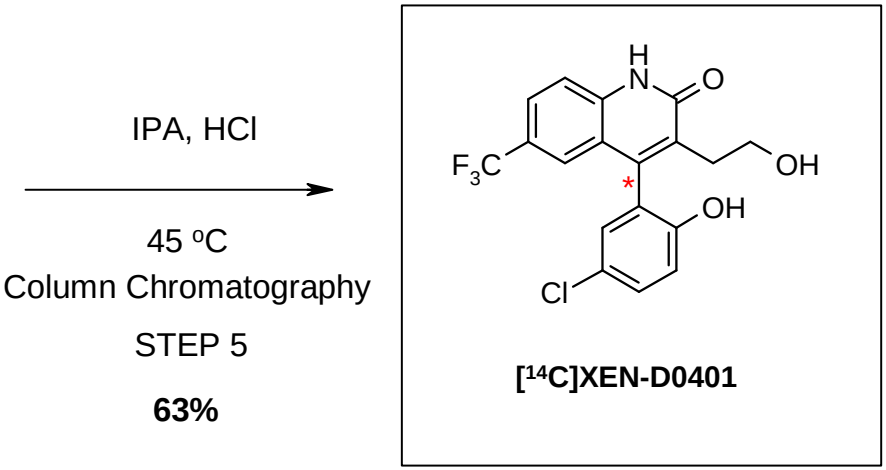

NH2
CF3
Cl
O
STEP 1
NHCOt-Bu
CF3
n-BuLi, Hex, THF
-78 oC
O
Li
N
Li
CF3

(**5**) **83%** (**6**)


MeO
O
*
OMOM
Cl
[14C]-4
Column chromatography
STEP 2
71%
NHCOt-Bu
O
F3C
*
OMOM
Cl
[14C]-7

S L Kitson, S J Jones *et al*. *J Label Compd. Radiopharm* **2010**, 53, 140-146

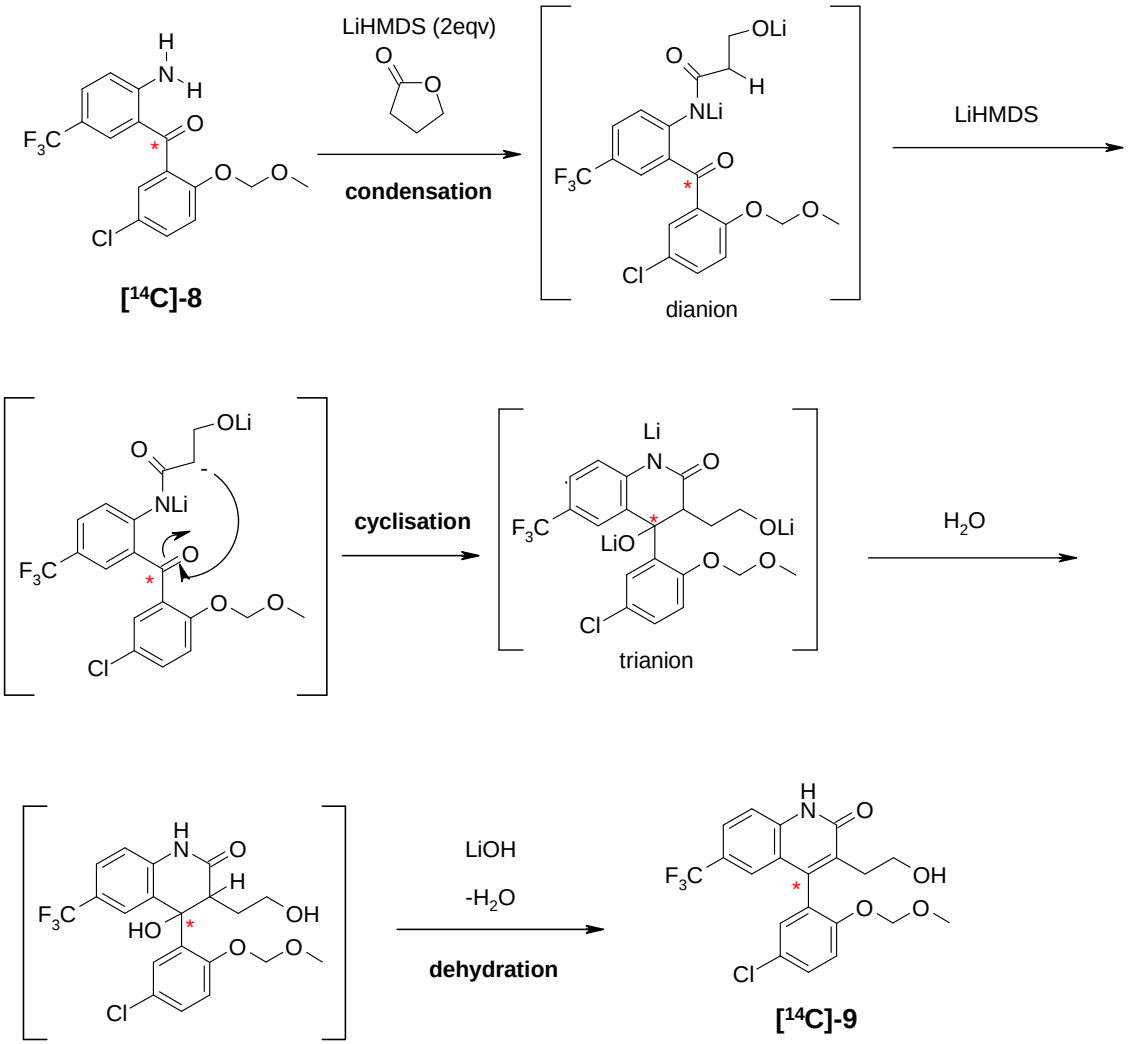




Overall Radiochemical Yield ~ 30%

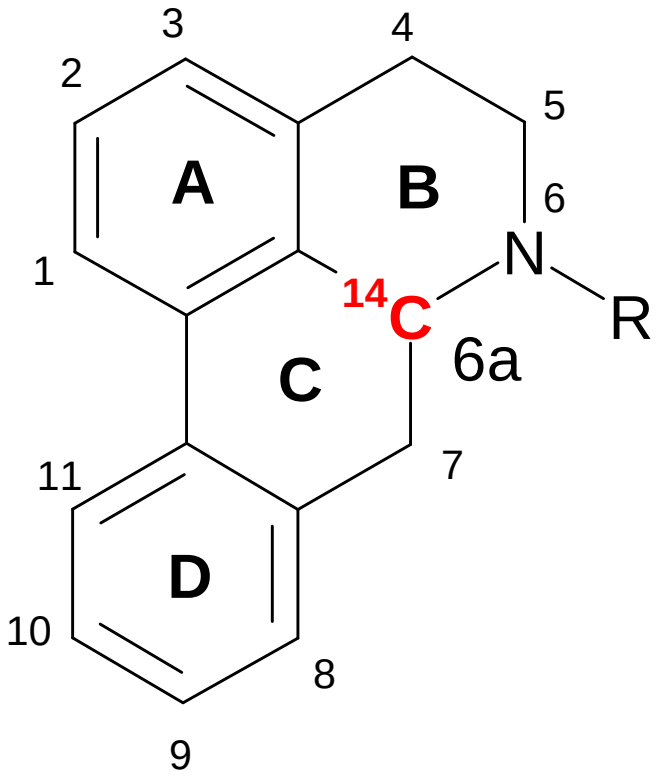
* = Carbon-14 label

Key Step: A one pot construction of the quinolin-2-one ring under strong base



Aporphine skeleton ‘naturally occurring’

Dicentra formosa
'Bleeding heart'



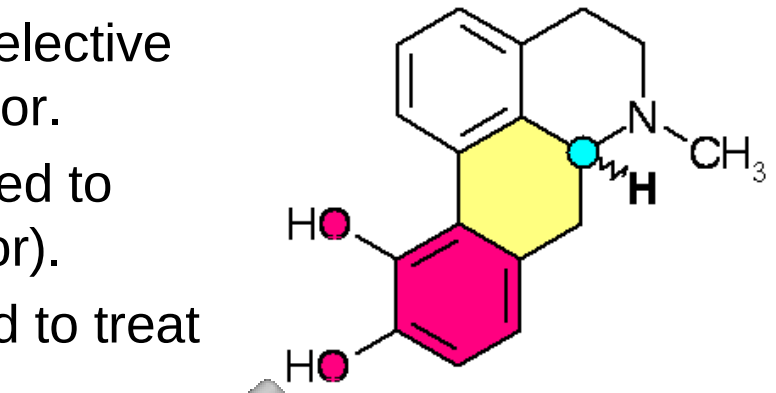
S L Kitson. *J Label Compd Radiopharm;* ***2007****, 50,* 290-294

S L Kitson. *J Label Compd Radiopharm;* **2006**, 49, 517-531

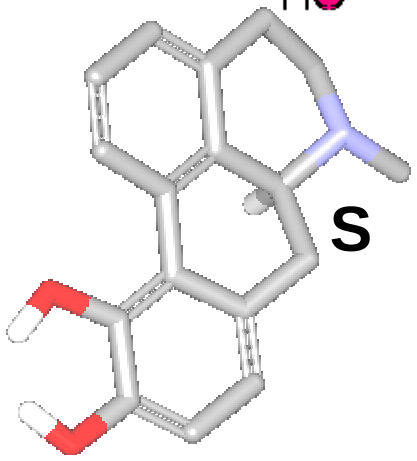
Structure & Function of (*R*)-(-)-Apomorphine

- (***R***)-(-)-Apomorphine is a potent non-selective D_1/D_2 agonist at the dopamine receptor.
- (***R***)-(-)- Apomorphine (Apokyn™) is used to control Parkinson's disease (**D_2** receptor).
- (***R***)-(-)- Apomorphine (Uprima®) is used to treat erectile dysfunction (**D_1** receptor).

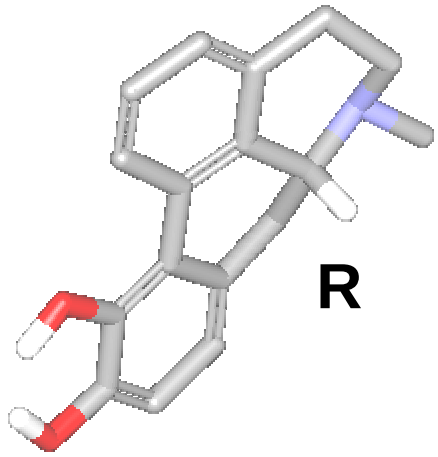
- **Features:**
- Contains an *ortho*-phenol motif.
- Tetracyclic carbon skeleton.
- Tetrahydroisoquinoline nucleus.
- 'Planar' structure.
- Contains an embedded dopamine pharmacophore.



(R/S)

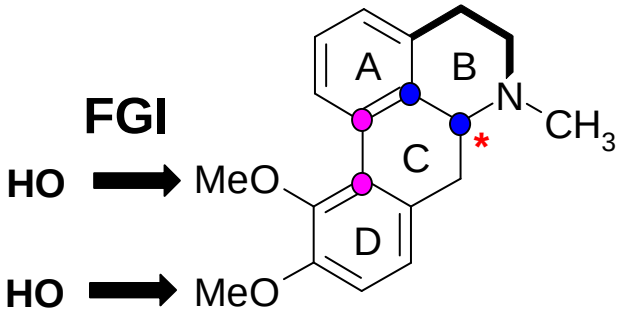


S



R

Retrosynthesis of Apomorphine

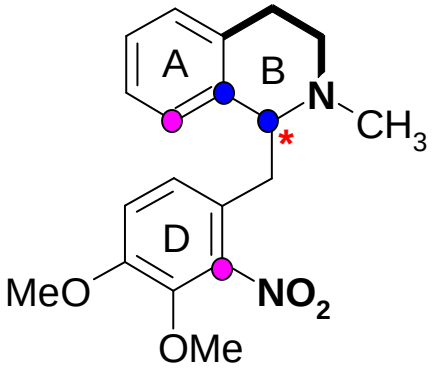


(*R/S*)-[6a-^{14}C]Apomorphine dimethyl ether

* = ^{14}C

RING C FORMATION

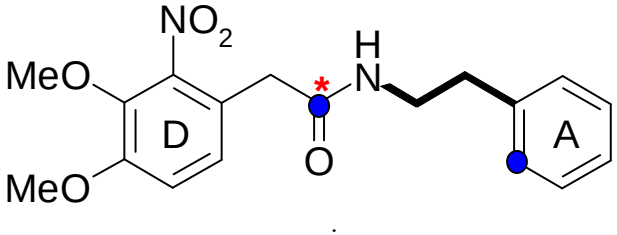
Pschorr coupling



Tetrahydro[^{14}C]isoquinoline

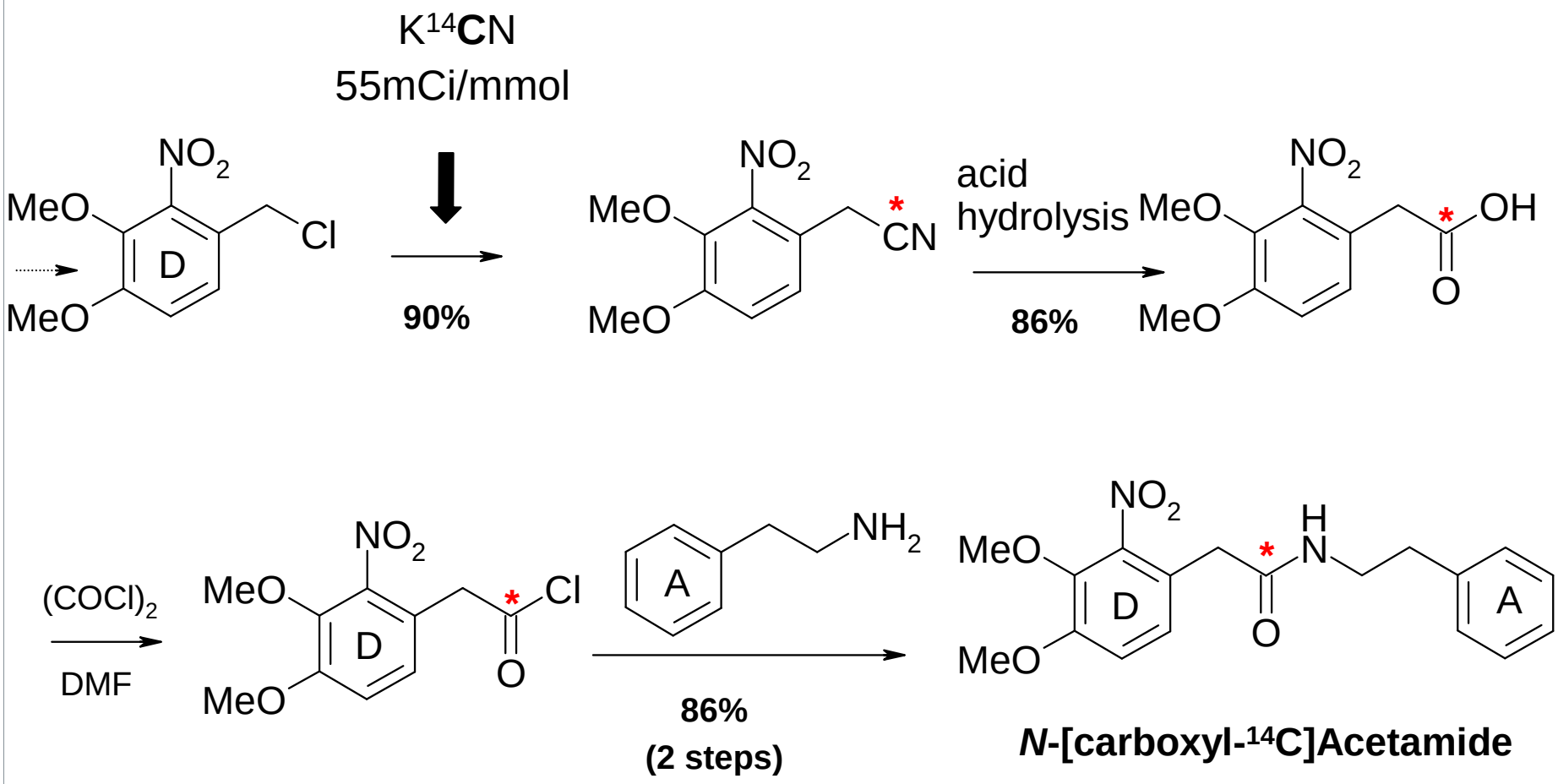
Bischler-Napieralski cyclodehydration

RING B FORMATION



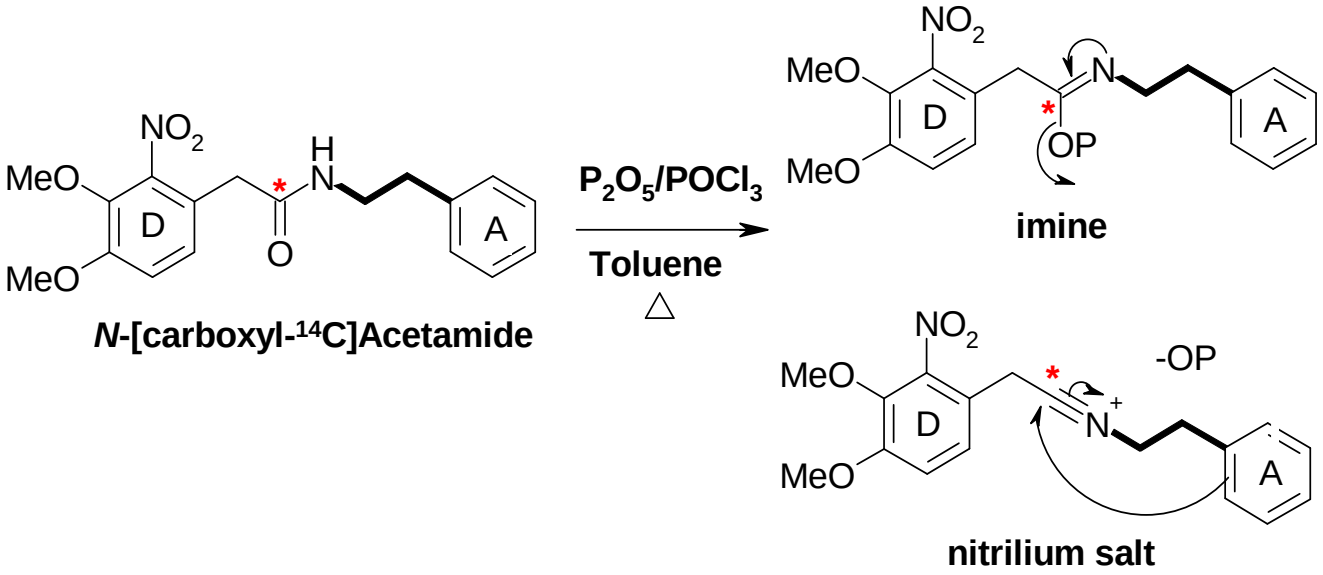
N-[carboxyl-^{14}C]Acetamide

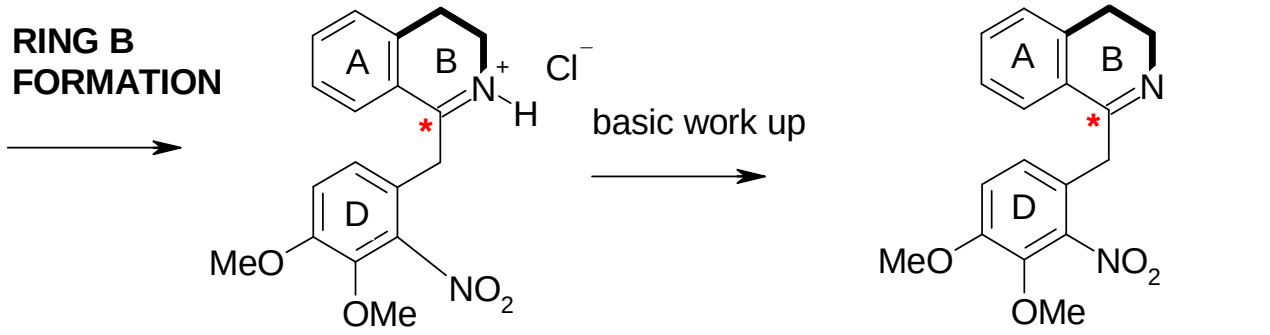
Synthesis of *N*-[carboxyl-^{14}C]acetamide



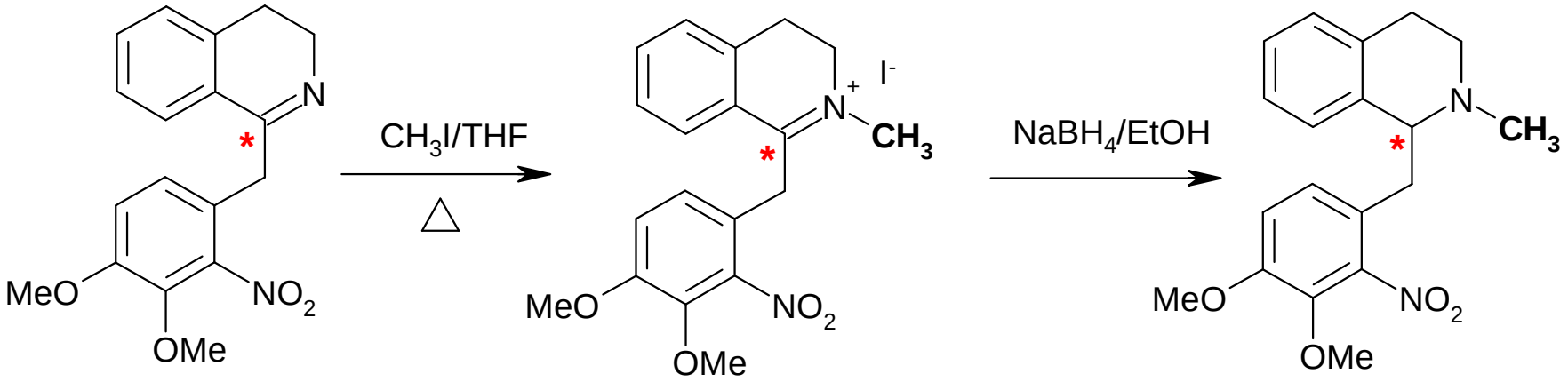
***N*-[carboxyl-^{14}C]Acetamide**

Bischler-Napieralski Cyclodehydration





N-Methylation Reduction Step



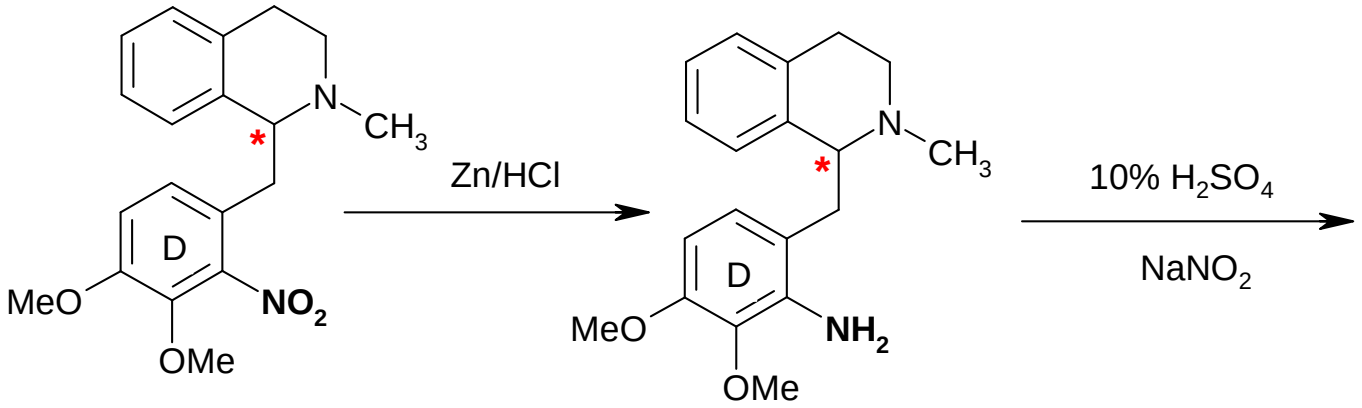
Dihydro[^{14}C]isoquinoline **[^{14}C]Methiodide** **Tetrahydro[^{14}C]isoquinoline**

Radiochemical yield = 77%

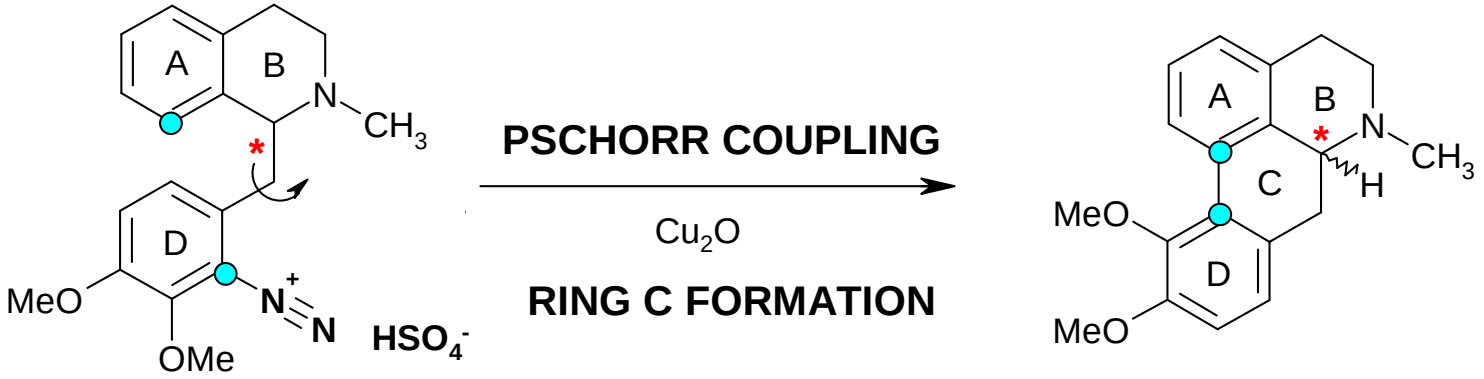
Bischler-Napieralski

Endocyclic Product

Reduction-Pschorr Coupling



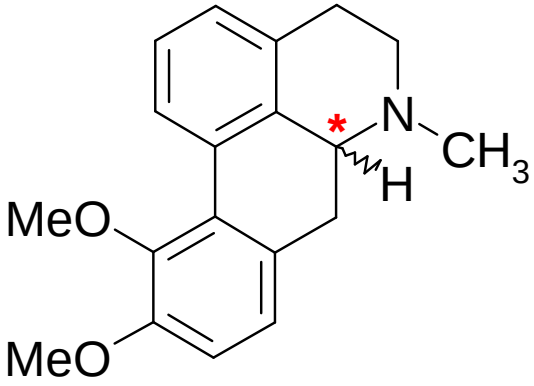
Tetrahydro[^{14}C]isoquinoline



aryldiazonium salt

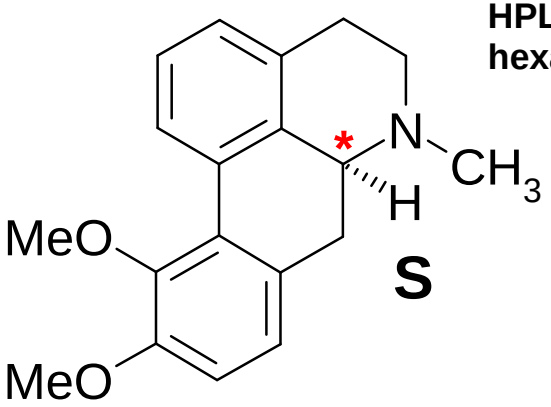
(*R/S*)-[6a-^{14}C]Apomorphine dimethyl ether

Chiral Separation



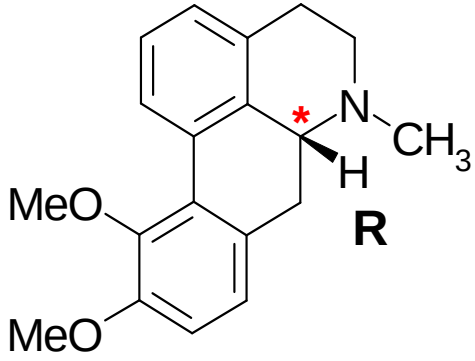
(*R/S*)-(+/-)-[6a-^{14}C]Apomorphine dimethyl ether

HPLC Conditions: Chiralcel OD column, eluting with hexane/propan-2-ol/dimethylamine [950:50:5]



(*S*)-(+)-[6a-^{14}C]Apomorphine dimethyl ether

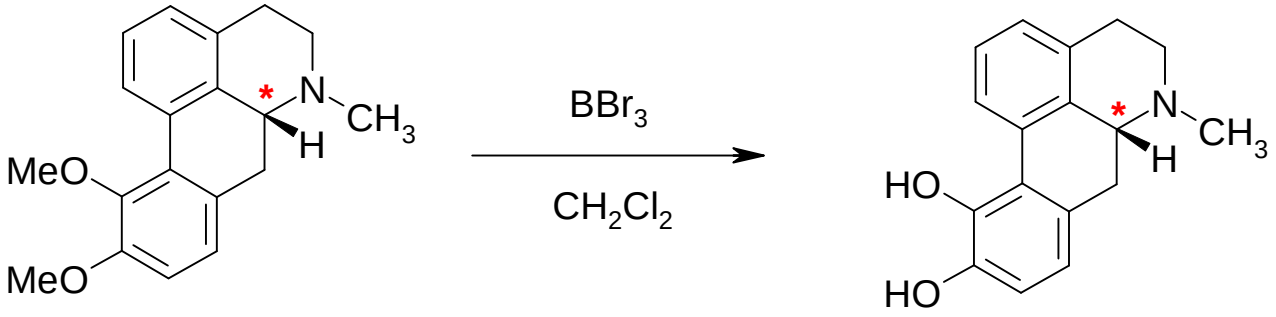
Radiochemical yield = 11% from Tetrahydro[^{14}C]isoquinoline



(*R*)-(-)-[6a-^{14}C]Apomorphine dimethyl ether

Radiochemical yield = 16% from Tetrahydro[^{14}C]isoquinoline

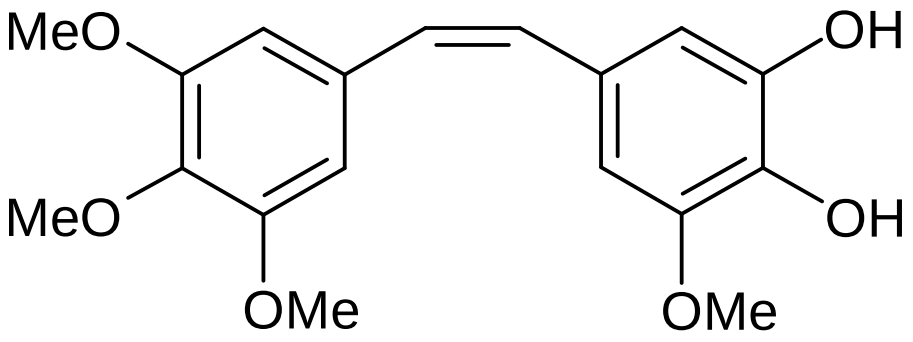
(*R*)-(-)-[6a-^{14}C]Apomorphine



(*R*)-(-)-[6a-^{14}C]Apomorphine dimethyl ether **(*R*)-(-)-[6a-^{14}C]Apomorphine**

- PLRP-S column (CH_3CN:HCl aq)
- Specific Activity: 55mCi/mmol
- Radiochemical purity => 98%
- Radiochiral purity => 99%

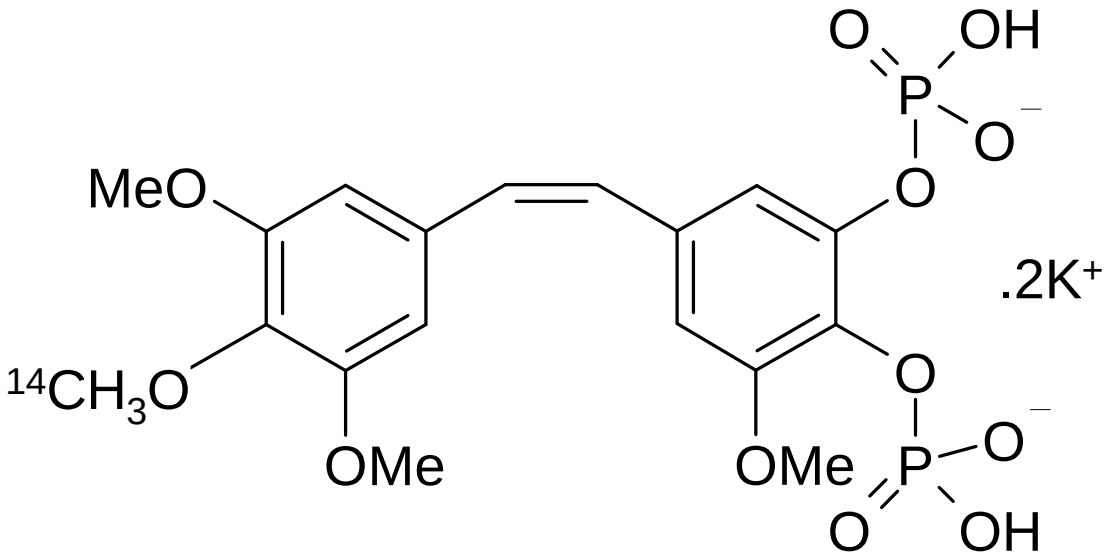
Combretastatin A-1



- **Combretastatin A-1 is a natural product first isolated from the African bush willow tree in the 1980s**

- **Chemotherapeutic properties by acting on the tumour vasculature, leading to blood flow shut down and ultimately tumour death**

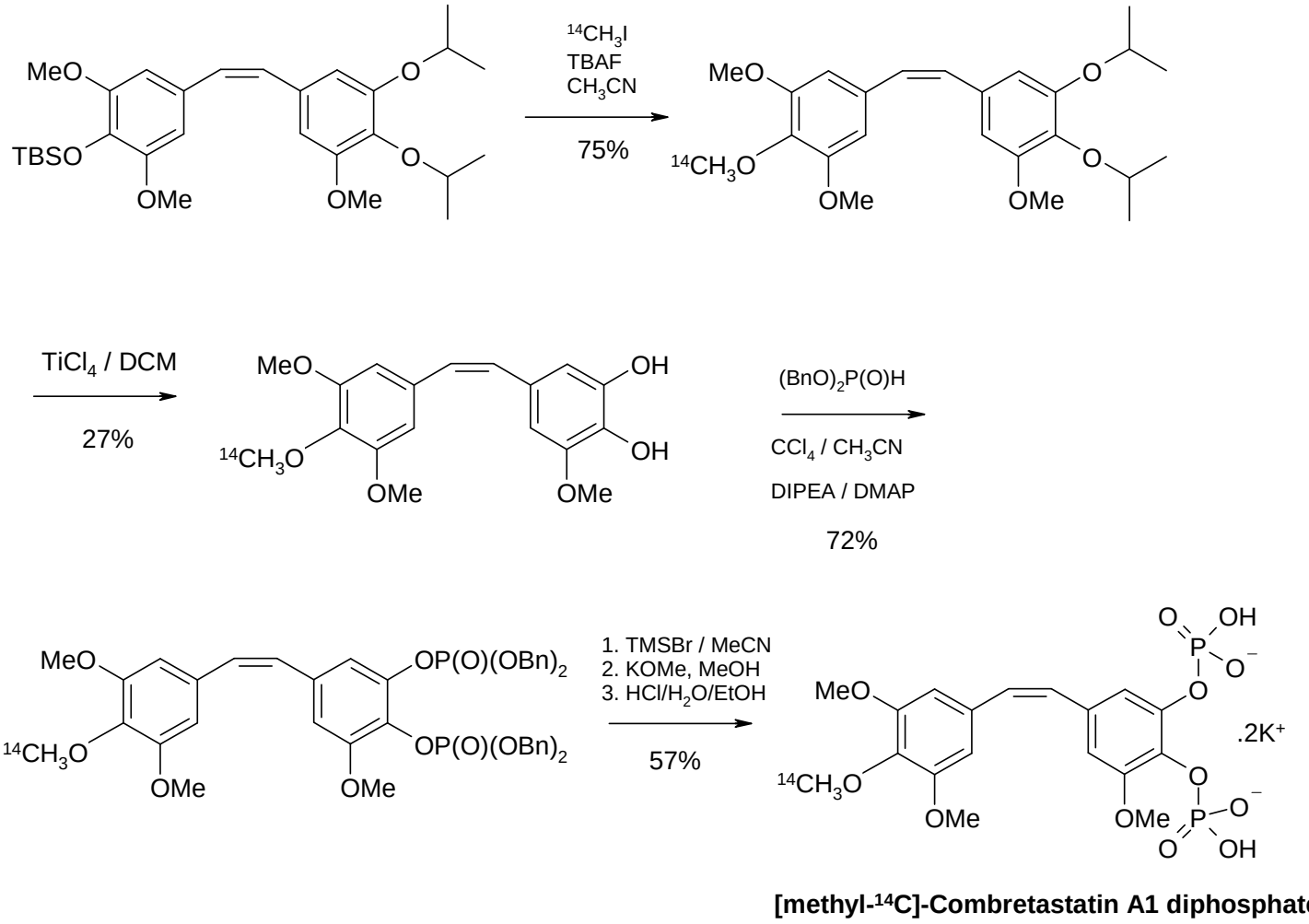
Target: [methyl-^{14}C]Combretastatin A-1 diphosphate



- Treatment of solid tumours
- The pro-drug undergoes *in vivo* de-phosphorylation *to* generate the active agent combretastatin A-1

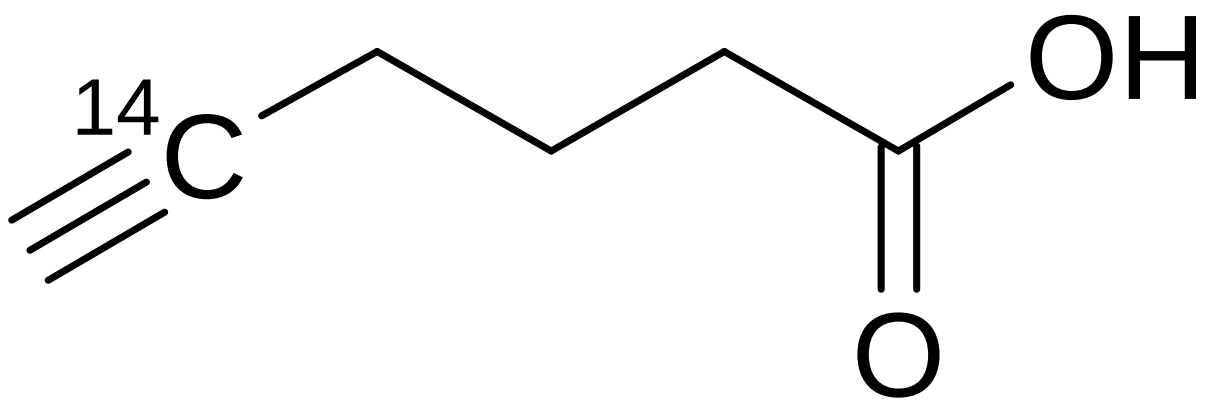
^{14}C Synthetic route

R T Brown *et al*. *J Label Compd. Radiopharm* **2009**, 52, 567-570



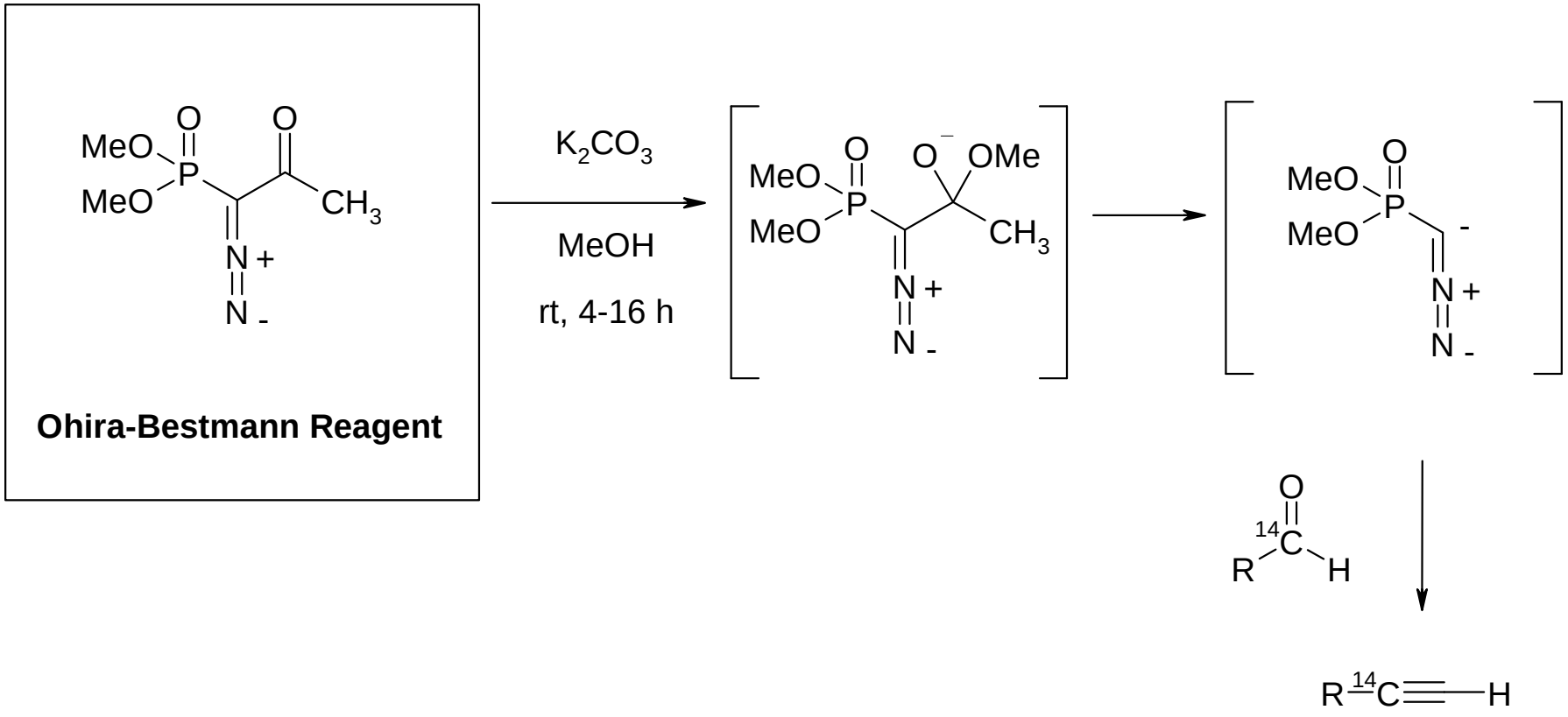
[methyl-^{14}C]-Combretastatin A1 diphosphate

[^{14}C]Acetylenes

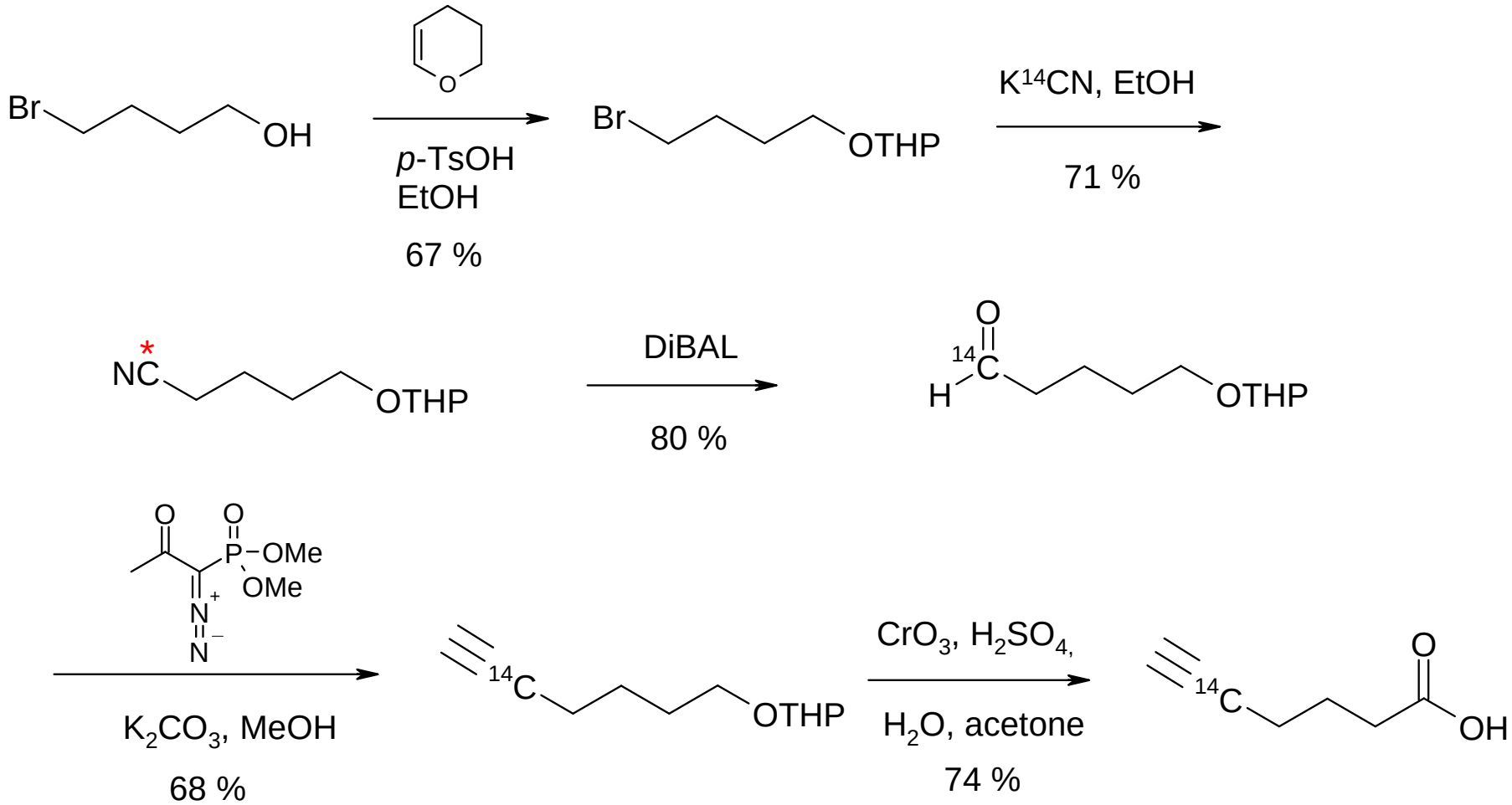


Target: [5-^{14}C]Hex-5-ynoic acid

[^{14}C]Acetylenes:
Ohira Bestmann Reagent



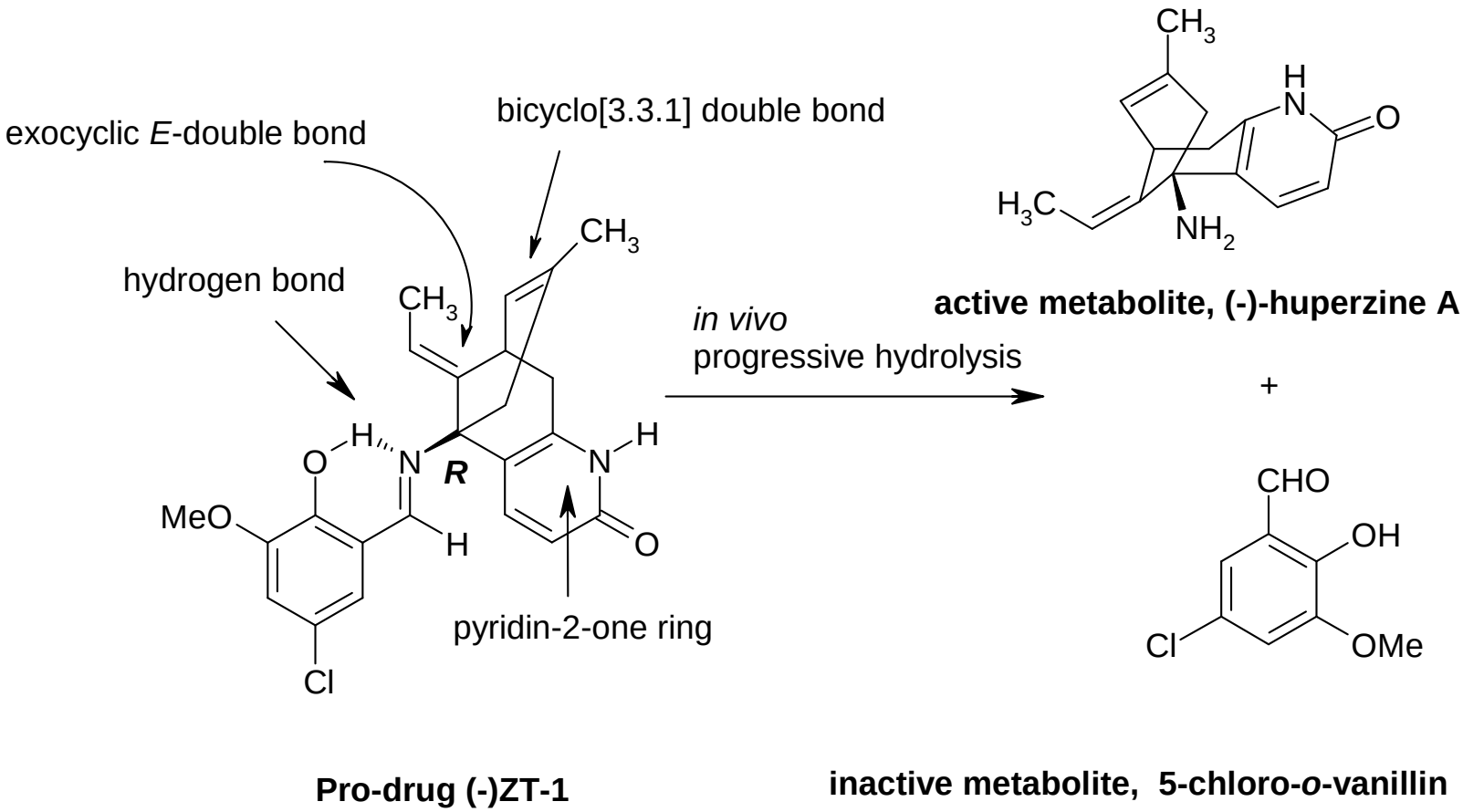
^{14}C Acetylene Synthesis



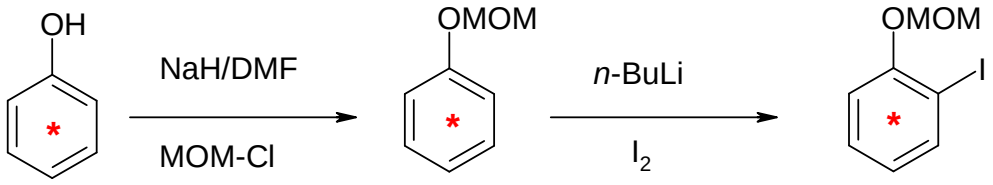
Overall Radiochemical Yield 29 % from [^{14}C]KCN

[^{14}C]ZT-1: a new generation of acetylcholinesterase (AChE) inhibitors

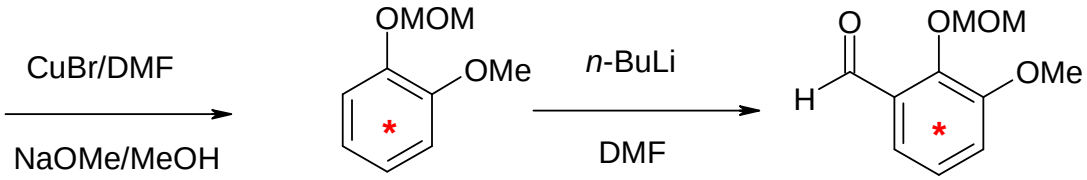
L Leman, S L Kitson *et al. J. Label Compd. Radiopharm* **2011**, 720-730



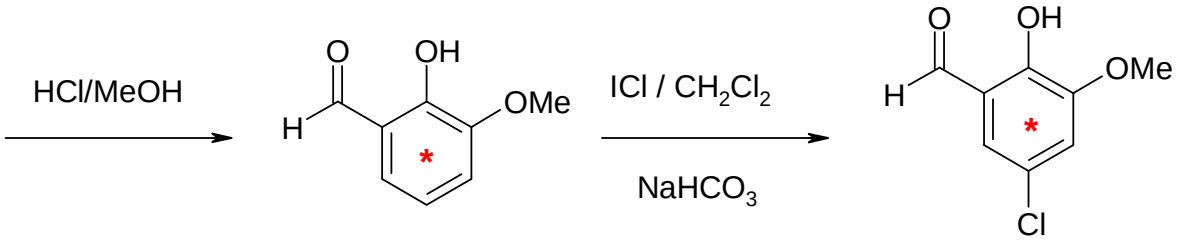
^{14}C Synthesis



[^{14}C]-1 **Step 1** **[^{14}C]-2** **Step 2** **[^{14}C]-3**



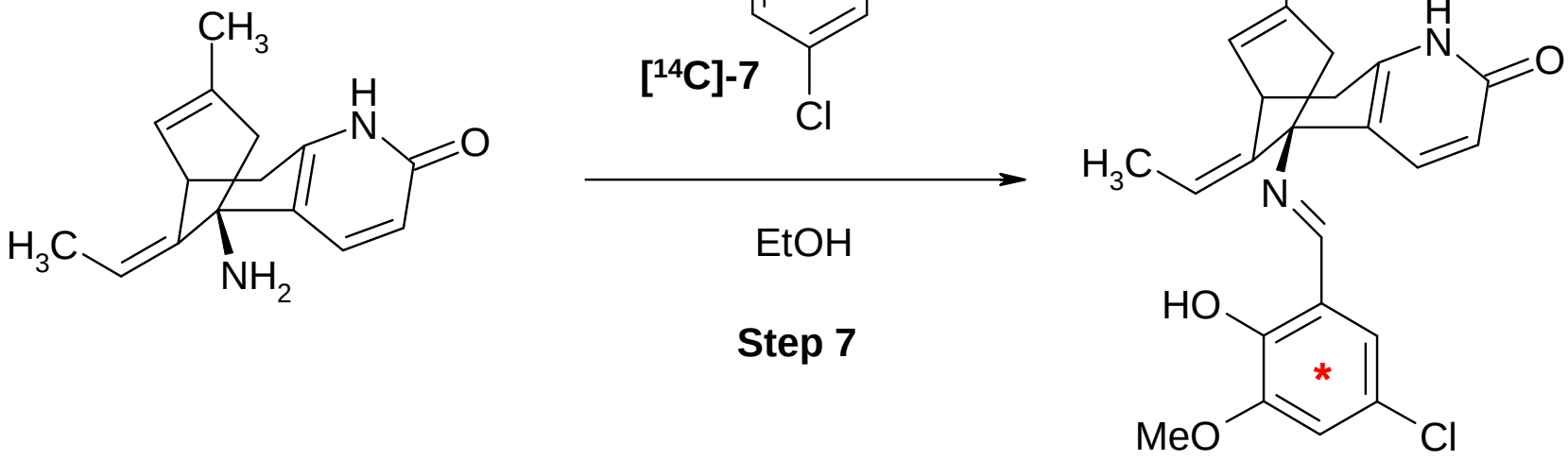
Step 3 **[^{14}C]-4** **Step 4** **[^{14}C]-5**



Step 5 **[^{14}C]-6** **Step 6** **[^{14}C]-7**

* = U-^{14}C

[^{14}C]-ZT-1



(-)-Huperzine A

[^{14}C]-7

[^{14}C]-ZT-1

Conclusion

When designing a ^{14}C labelled synthesis it is important to consider the following:

- **Identify** simple starting materials from the barium ^{14}C carbonate ‘staircase’ which are commercially available or alternatively easily made

- **Plan**, develop and execute the synthetic methodology to the final drug substance. This approach can often restrict the position of the label in the drug and will cause a change in the drug purity profile from the original laboratory synthesis route

- **Locate** a biologically stable position for the ^{14}C label

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