"The methyl group, so often considered as chemically inert, is able to alter deeply the pharmacological properties of a molecule."
-Camille Wermuth

The earliest recorded use of methanol is from the ancient Egyptians who used it in their embalming process

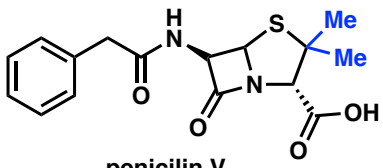
In 1661, Robert Boyle isolated and described methanol, distilling it from the bark of Boxwood trees

Jean-Baptiste Dumas and Eugene Peligot determined the chemical structure of methanol in 1834

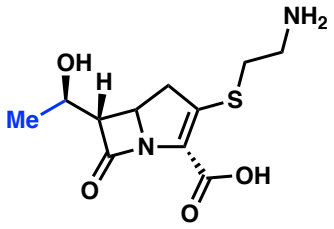
Dumas and Peligot formed the word "methylene" during their attempt to describe methanol.
Methylene came from the Greek *methy* (wine) and *hȳlē* (wood) to mean "alcohol from wood" but they misused the term *hȳlē* which translates more closesly to "forest." The proper term would have been *xylo.*

Nonetheless, in the 1840s, the word "methyl" was derived from their term "methylene"

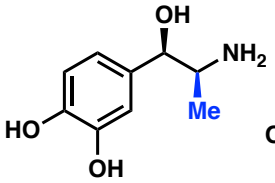
Methylated Natural Products



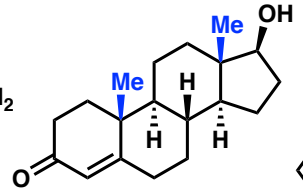
penicillin V
Sheehan, 1957 is



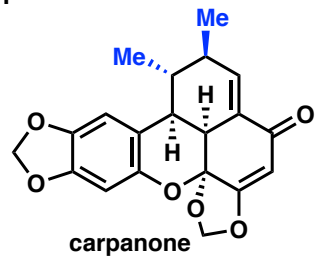
thienamycin
Merck, 1980



vitamin B_1
Cline 1936



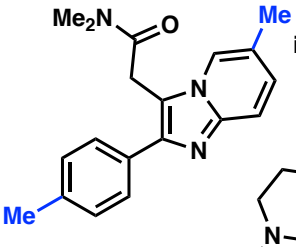
testosterone
Johnson, 1955



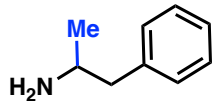
carpanone
Chapman, 1971

Methylated Drugs

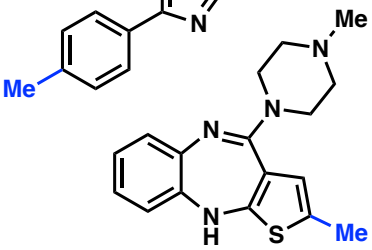
Of the top 200 pharmaceutical products by retail sales in 2018, over 73% of the small-molecule drugs contain at least one methyl group,



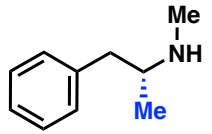
Ambien (zolpidem)
insomnia treatment



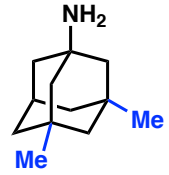
Adderall (amphetamine)
ADHD and narcolepsy treatment



Zyprexa (olanzapine)
antipsychotic medication



L-methanphetamine
nasal decongestant



Namenda (memantine)
dementia treament

Overview

1. Methyl Effects on Drug Efficacy
2. Hot Spots for Methyl Effects
2. Biological Methylation
3. Synthetic Methods: sp^2, sp^3, and sp C-H Methylations
4. Late Stage Methylations

Disclaimer: This is certianly not a comprehensive review of methylative methods.

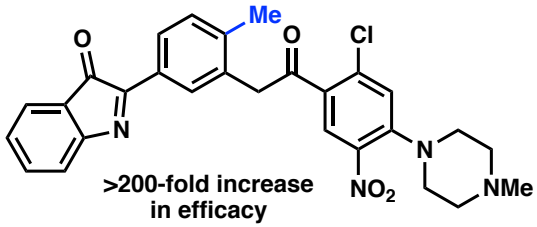
Topics Not Covered
-> N-, S-, or O-methylations
-> Methylations from activated C-X bonds, including cross-couplings
-> Installation of "hidden" methyl groups

Methyl effects are not: homologation or esterification / etherification

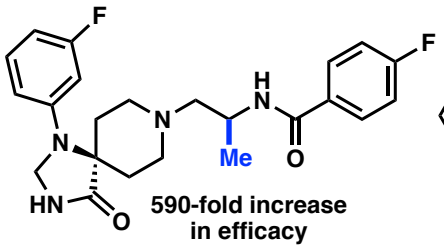
Relevant Literature

Methyl Effects in Med Chem: Chem. Rev. 2011, 111, 5215; Angew. Chem. Int. Ed. 2013, 52, 12256
Methyl Effects on Protein-Ligand Binding: J. Med. Chem. 2012, 55, 4489
Transition [M]-catalyzed Methylations: Adv. Synth. Catal. 2015, 357, 1333
Peroxides as Methylating Reagents: Synthesis 2016, 48, 329
Radical SAM-mediated Methylations: J. Bio. Chem. 2015, 290, 7, 3995
Late Stage Functionalization: Chem. Soc. Rev., 2016, 45, 546
Removal/Modification of Directing Groups in C-H Functionalization Chemistry: Org. Biomol. Chem. 2021, Advance Article
Borrowing Hydrogen Catalysis: Chem. Rev. 2018, 118, 1410

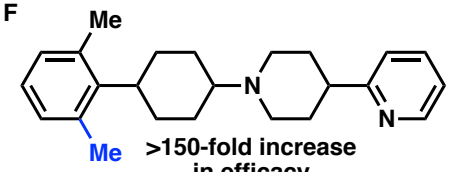
A single methyl can impart dramatic effects on biological activity



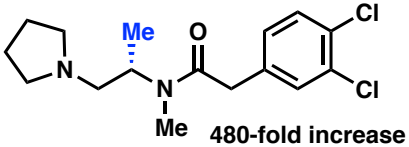
>200-fold increase in efficacy



590-fold increase in efficacy



>150-fold increase in efficacy



480-fold increase in efficacy

(compared to *des*-methyl drug targets)

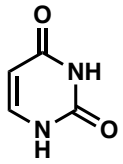
Ligand-Lipophilicity Efficiency (LLE) can be incredibly favorable for methyl, as it adds little lipophilicity while (potentially) having profound effects on potency

Compare to CF_3, which may increase potency, but deleteriously affects lipophilicity and therefore impacts a drug candidate's ADME (absorption, distribution, metabolism, and excretion) while methyl is less likely to negatively impact ADME factors

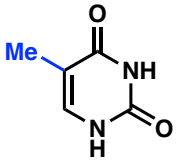
- > This dramamtic effect is extremely rare, but it can be so profoundly impactful that it is often worth testing potential methyl "hot spots" on the molecule, especially if the analogues are easily accesible

Angew. Chem. Int. Ed. 2013, 52, 12256

Uracil

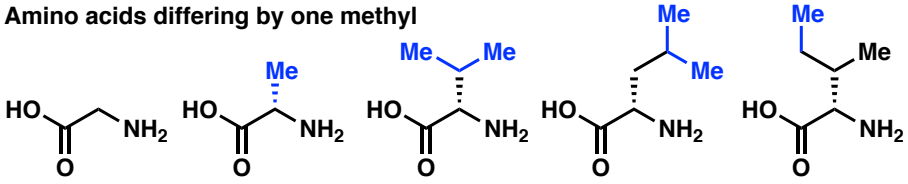


Thymine



DNA and RNA bases only differ by one methyl - impacting transcription and replication due to DNA conformation

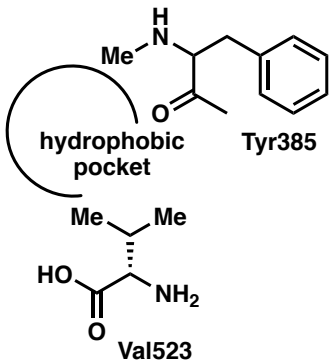
Amino acids differing by one methyl



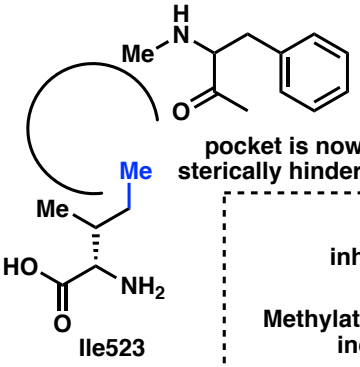
Prostaglandin endoperoxide synthases (PGHS) convert arachadonic acid to prostagladins

This difference in hinderance allows for the development of selective NSAIDs

PGHS-2



PGHS-1

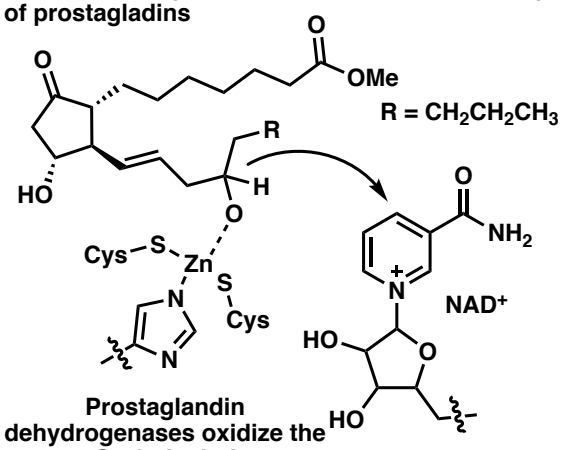


Inhibition of PGHS-2 is primarily responsible for the analgesic effect of NSAIDs, as these receptors synthesize prostaglandins responsible for mediating pain, fever, and inflammation

Blocking PGHS-1 causes unwanted gastrointestinal side effects

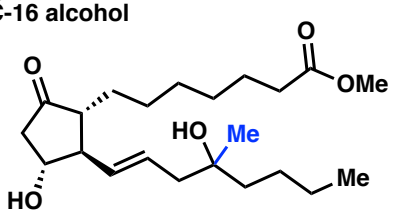
Celebrex (celecoxib) is a PGHS-2 selective NSAID, brought to the market in 1999

Common methylation tactic to increase efficacy of prostagladins



Prostaglandin dehydrogenases oxidize the C-16 alcohol

misoprostol inhibits gastric secretion



Methylation at C-16 blocks hydride transfer leading to [O] and thus increases the drug's half-life and reduces side effects

The Methyl Behind the Curtain

It's Not Magic, It's Just….

Change in Desolvation Energy

Due to its slight increase in lipophilicity (ΔclogP = 0.55 per methyl), methylation lowers the free energy of solvation in hexadecane

When transferring a solvent exposed drug to a hydrophobic protein pocket, there is an energy benefit, measured as ΔG_{trans}

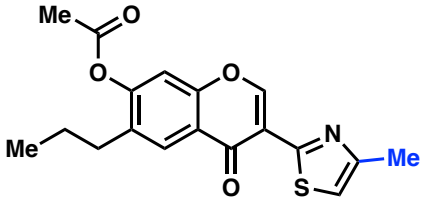
Proton to methyl replacement has a benefit of $\Delta\Delta G_{trans}$ = ~0.7 kcal/mol

chromone12

Antagonist of Adenosine A_{2a} Receptor

33-fold better binding affinity than *des*methylated drug lead

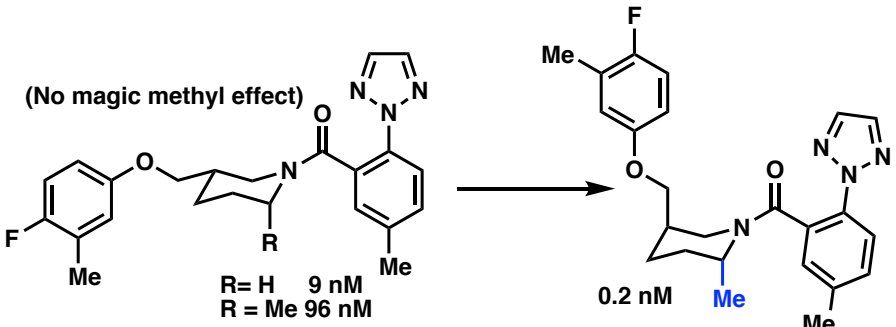
The methyl displaces a high energy water molecule from the active site



In Silico Pharmacol. 2013, 1, 23

Productive Change in Conformation

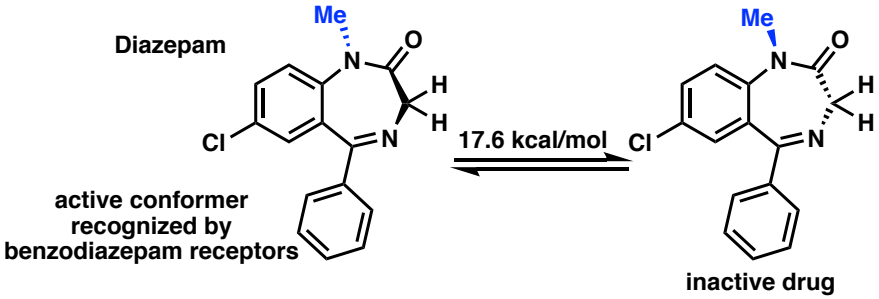
Lock molecule into conformation that more closely resembles its conformation when bound to a protein, lowering the reorganization energy



Notably, *cis* orientation of the substituents has deleterious effect on efficacy compared to *des*methylated drug lead

ChemMedChem 2012, 7, 415
J. Med. Chem. 2012, 55, 4489
Bioorg. Med. Chem. Lett. 2009, 19, 2997

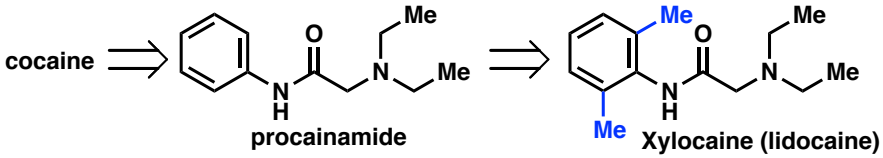
Methyl induces high energy barrier between atropisomers



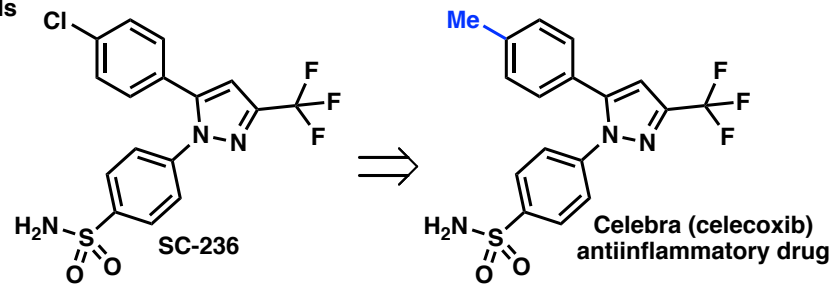
Methylene hydrogens have a coupling constant of 14.0 Hz

*Des*dimethyldiazepam has a low barrier of rotation (methylene hydrogens are present as a singlet in ^{1}H NMR)

Effect on Drug Metabolism



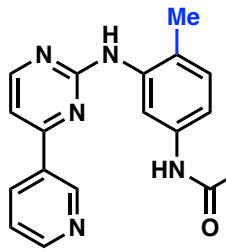
Lidocaine, an antiarrhythmic drug and anesthetic, was iteratively designed from cocaine
Procainamide, an earlier derivative, is active but metabolized quickly *in vivo*
Methyls on lidocaine twist arene out of plane and this increased steric bulk prevents it from binding to plasmatic amidases which hydrolyze amide bonds



SC-236 has a half life of 117 h as the chlorine protected metabolism by P450s
A methyl group provided the needed lipophilicity in the C-4 position on the arene for proper selectivity while, reducing the half-life to 17 h

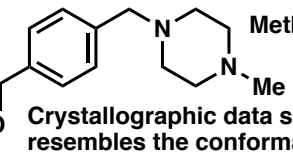
Hot Spots for Magic Methyl Effects

1. *Ortho* to Large substituents



Gleevec (imatinib)
4-fold increase in potency

Treatment for chronic myeloid leukemia

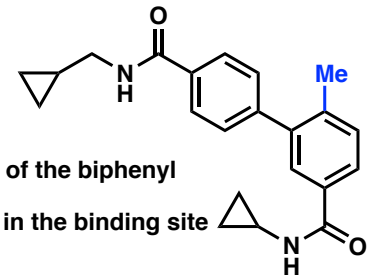


Methyl group increased binding in the Abl1 kinase pocket

Crystallographic data shows that imatinib more closely resembles the conformation of the bound drug than the *des*-methyl derivative

α-p38 MAP kinase inhibitor
>200-fold increase in efficacy

Blocks pathways to inflammatory responses

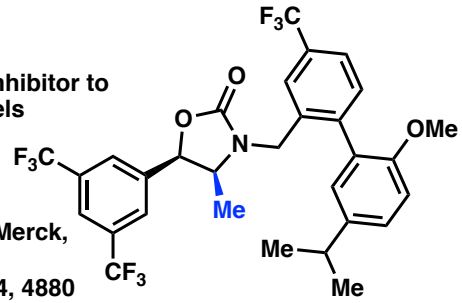


Ortho methylation changes the dihedral angle of the biphenyl system, lowering the preorganization energy
The methyl group also fills a lipophilic pocket in the binding site

2. On Substitued Rings

Anacetrapib

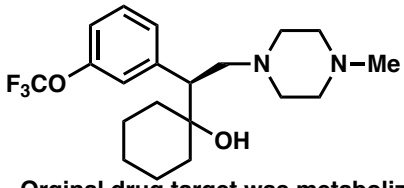
Chloesteryl ester transfer protein inhibitor to reduce HDL cholesterol levels

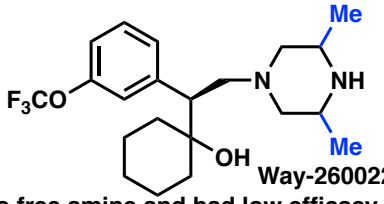


6-fold increase in efficacy
Compound displayed favorable ADME parameters

Advanced to phase 3 clinical trials at Merck, but failed in 2017

J. Med. Chem. 2011, 54, 4880

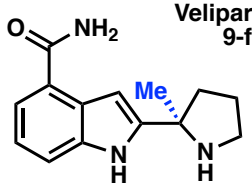




Orginal drug target was metabolized to free amine and had low efficacy
Dimethylation increased metabolic stability

Way-260022

Pfizer is developing as treatment for regulation of blood vessel constriction and as a norepinephrine reputake inhibitor



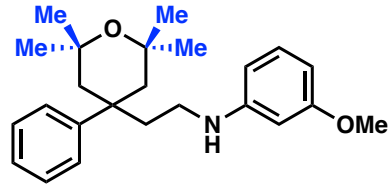
Veliparib
9-fold increase in potency

Methyl group thought to impart favorable pharmacokinetics
AbbVie is developing as an anti-cancer drug which prevents cancer cells from repairing by inhibiting polymerases

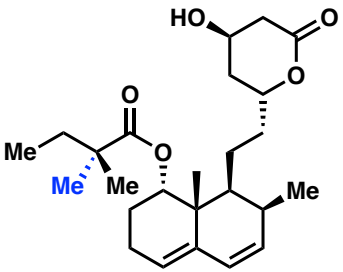
> 595-fold increase in potency

Displays anti-cancer activity by inhibiting isoprenylcysteine carboxyl methyltransferase (ICMT)

J. Med. Chem. 2011, 54, 5031



3. Between Freely Rotating Bonds



Zocor (simvastatin)
*des*methyl: lovastatin

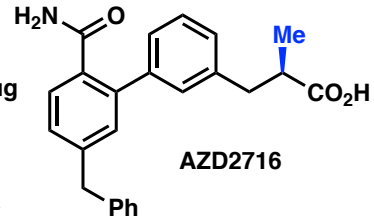
Simvastatin is one of the most prescribed drugs in the world, used to treat high cholesterol
~ 2.5-fold increase in potency

Methyl thought to fill lipophilic pocket in active site and potentially slow hydrolysis of ester, increasing half-life

Treatment of coronary artery disease by inhibiting secreted phospholipase A_2

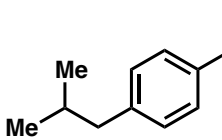
Des-methyl drug lead had negative drug-drug interactions when administered with statins
Steric bulk reduced off-target binding and yielded a 70-fold increase in efficacy

Currently being developed by AstraZeneca



AZD2716

ACS Med. Chem. Lett. 2016, 7, 884



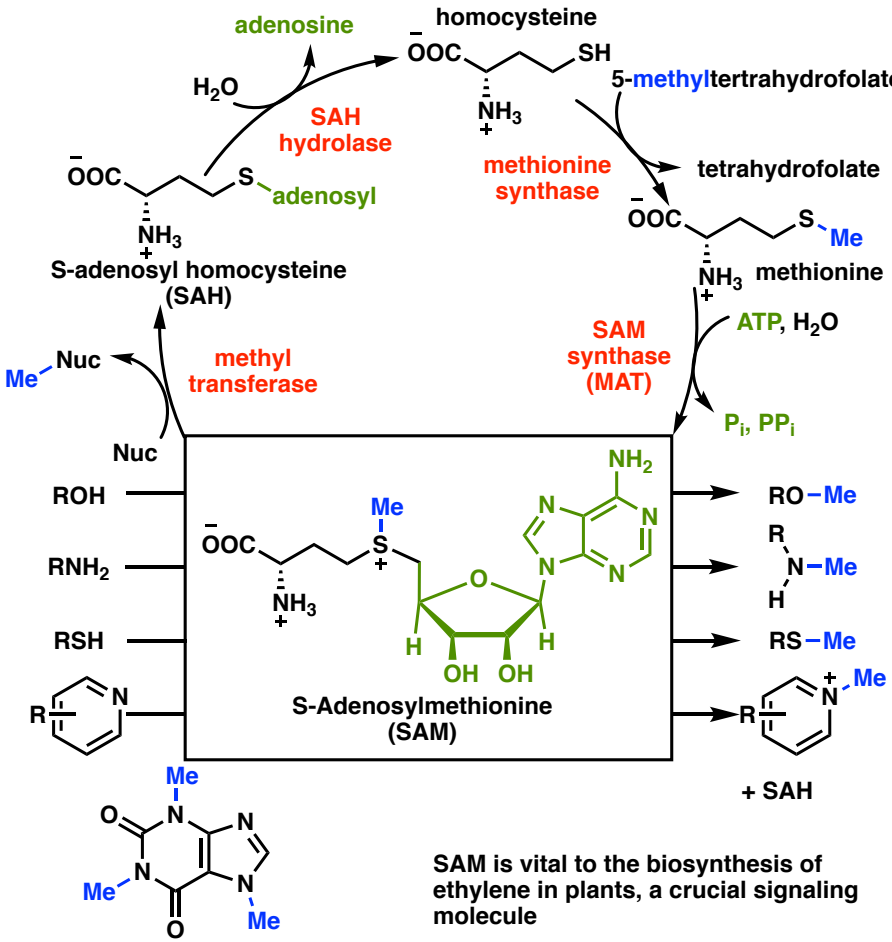
Ibuprofen

ibufenac, the *des*methyl derivative, was also marketed as an NSAID but was taken off the market in 1967 for hepatotoxicity (damage to liver cells)
The methyl on Ibuprofen slows the metabolism of the drug, increasing its half-life from 1.1 h to ~4 h

Bioorg. Med. Chem. Lett. 2018, 28, 3283

S-Adenosylmethionine "A Natural Methyl Iodide"

Primary Methyl Donors in Biological Systems



3 equivalents of SAM are used to produce caffeine from adenine, the 3 methyl groups make it a particularly strong CNS stimulant

SAM is vital to the biosynthesis of ethylene in plants, a crucial signaling molecule

SAM methylates cytosine to 5-methyl cytosine in DNA methylation

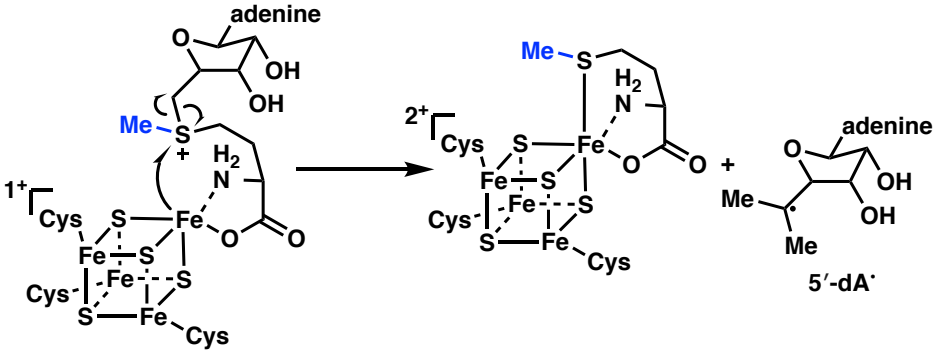
SAM is implicated in cyclopropanations, including in jawsaymicin biosynthesis

Chem. Rev. 2011, 111, 5215
FEBS Lett. 2001, 499, 50
PNAS 2010, 107, 19, 8537
J. Biol. Chem. 2015, 290, 7, 3995

SAM is a cofactor in the C-H methylation of many sp^3 and sp^2 bonds, including the bleomycin family of antiobiotics, thiostrepton, and nosiheptide among other antiobiotics, as well as polytheonamide

These C-H methylations consume two equivalents of SAM

The first equivalent of SAM undergoes a redox event using radical SAM (RS) enzymes, where redox active $4Fe/4S^{1+}$ clusters cleave SAM to 5′-deoxyadenosyl 5′-radical (5′-dA·)

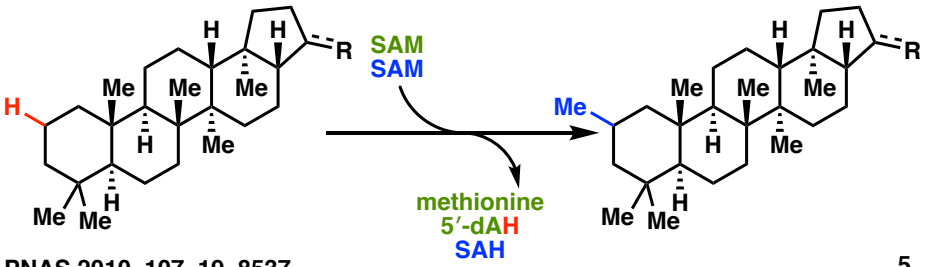


5′-dA· will then act as a hydrogen abstractor on the substrate to be methylated

The second equivalent of SAM will then be attacked by the newly formed radical, homolytically cleaving the Me-S bond

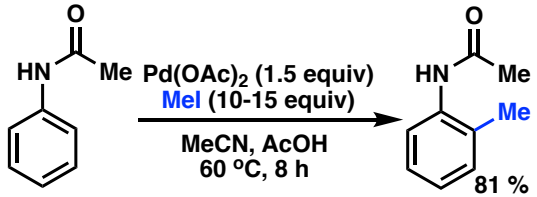
Chem. Rev. 2014, 114, 8, 4229
Acc. Chem. Res. 2012, 45, 4, 555

hopane methylation requiring two equivalents of SAM



PNAS 2010, 107, 19, 8537

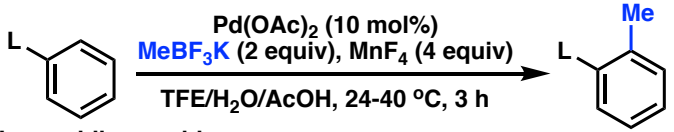
sp^2 Methylation



Catalytic turn over observed with an excess of acetanilide and AgOAc
AgOAc acts as stiochiometric oxidant
No formation of a Pd^0 species - catalytic turnover without reoxidation to Pd^{II} (milder conditions)

Realized the possibility of weakly coordinating directing groups to aid in catalytic C-H functionalization in acidic conditions

J. Am. Chem. Soc. 1984, 106, 5759

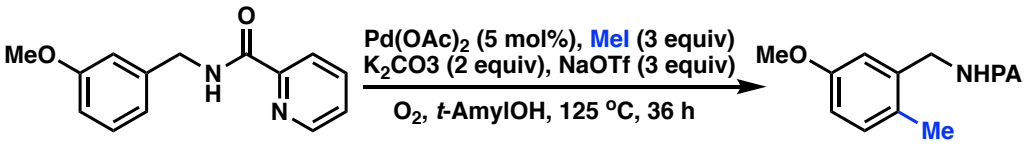


L = pyridine, amides

High valent (Pd^{III} or Pd^{IV}) pathway to avoid sluggish [O] to Pd^{II}
C-C coupling proceeds under mild conditions
No promoter or additives needed

Org. Lett. 2013, 15, 9, 2302

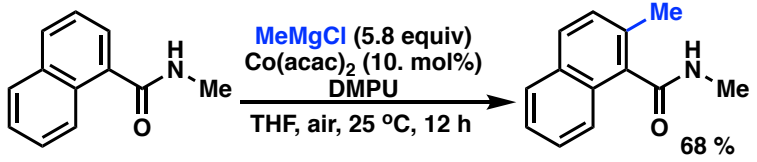
Picolinamide (PA)-directed Methylation



Silver free conditions, O_2 as sole oxidant
High regioselectivity controlled by bidentate ligand
DG removed under mild basic conditions

Org. Lett. 2011, 13, 18, 4850

Mild methylation conditions



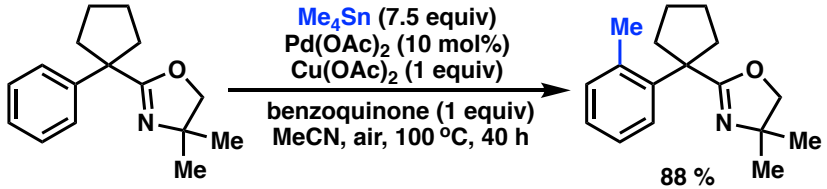
No high temp needed, air as [O]

Org. Lett. 2011, 13, 12, 3232

Organotin Reagents

First method for Pd^{II}-catalyzed alkylations of aryl C-H bonds with organotin reagents

Inherent problems: avoiding homocoupling of R_4Sn reagents and difficult to find conditions ammenable to each step of catalytic cycle

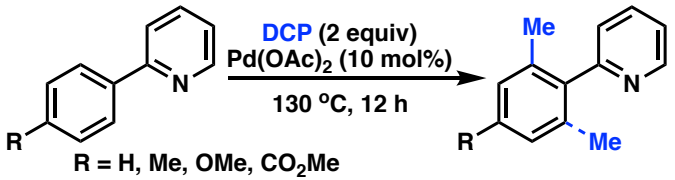


Non-conjugated directing group
Conjugated oxa group gives mixed mono- and di-substitution
Also uses a non-conjugated pyridine to direct

Benzoquinone to promote reductive elimination
Portionwise introduction of organotin reagent (slow reaction time)
Use of MW to lower reaction time to 10h

J. Am. Chem. Soc. 2006, 128, 78

First Direct Methylation of Aryl C-H bonds Using Dicumyl Peroxide



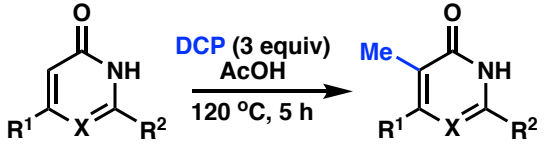
R = H, Me, OMe, CO_2Me

Peroxide serves as hydrogen acceptor and methylating reagent
Acetanalides also ammenable to conditions
Mixture of mono- and di- substituted products

J. Am. Chem. Soc. 2008, 130, 2900

Metal Free Conditions Using DCP

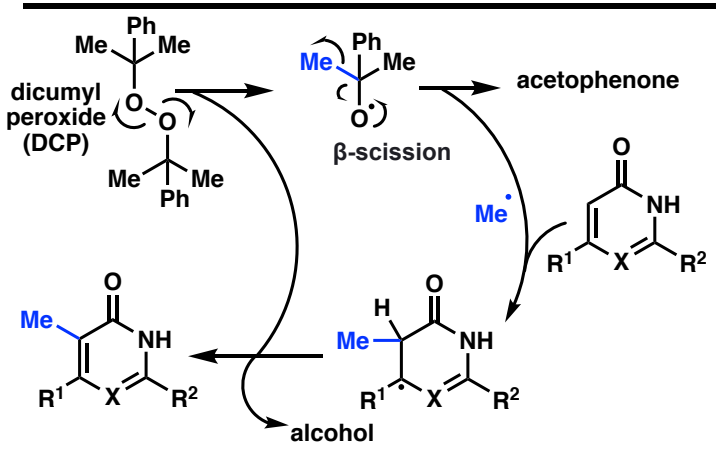
methylation of pyrimidinones and pyridinones



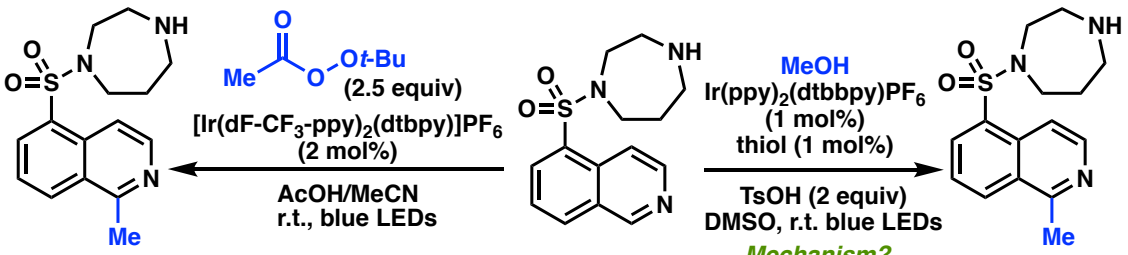
X = CH_2, N
$R^{1,2}$ = tolyl, phenyl, ArF, ArOMe, Me

63-96 %

Green Chem. 2017, 19, 919



Photoredox: Alcohol and Peroxide Motifs



Mechanism?

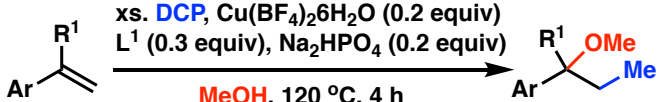
Regulates blood vessel constriction

No high temperature required
Basic amines, amides, alcohols, esters all tolerated

Nature, 2015, 525, 87
Angew. Chem. Int. Ed. 2014, 52, 4802

Methylative Difunctionalization of Alkenes Using Peroxides

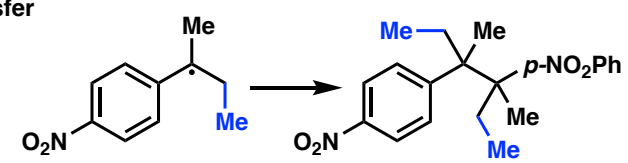
1,2-alkoxy methylation

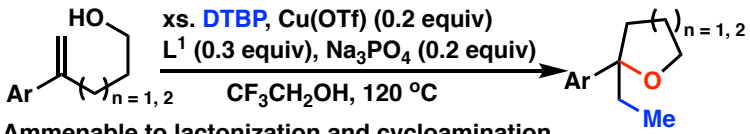


R^1 =. alkyl, aryl

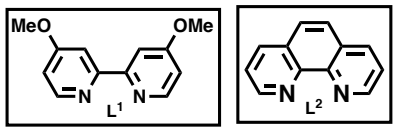
Ammenable to 1,2-azido methylation using LiN_3, $CuSO_4$, *t*-BuOH, L^2, and DTBP (di-*tert*-butyl peroxide)

Alkoxy methylation appears to go through radical-cation crossover mechanism, azido methylation a Cu redox azide transfer



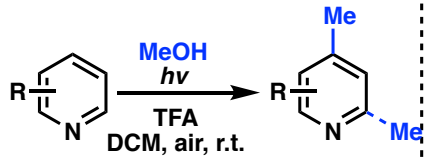


Ammenable to lactonization and cycloamination



Nat. Comm. 2018, 9, 3725

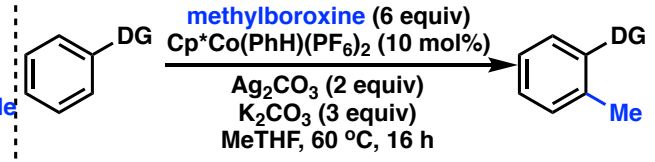
Metal Catalyst Free Methylations



Green, open flask
Tolerates esters, cyano, ethers
Five and six membered heterocycles

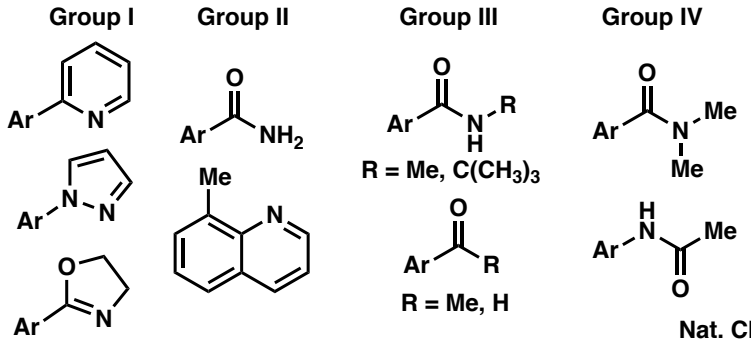
Chem 2017, 2, 5, 688

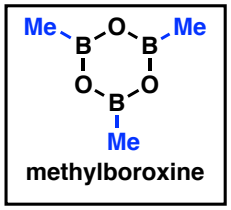
Methylation method for multiple directing groups



Conditions are ammenable to a broad range of directing groups including N-heterocycles, benzamides, amides

Allows for comparison of *directing ability*
Group I directs over III and IV
Group II and III direct over IV

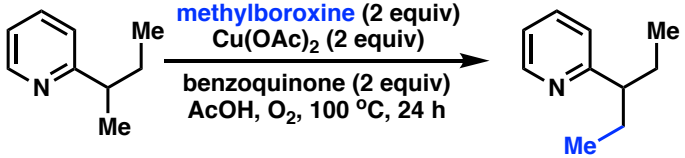


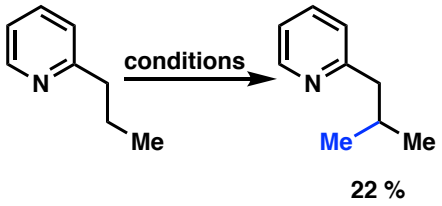


Nat. Chem. 2020, 12, 511

sp^3 Methylation

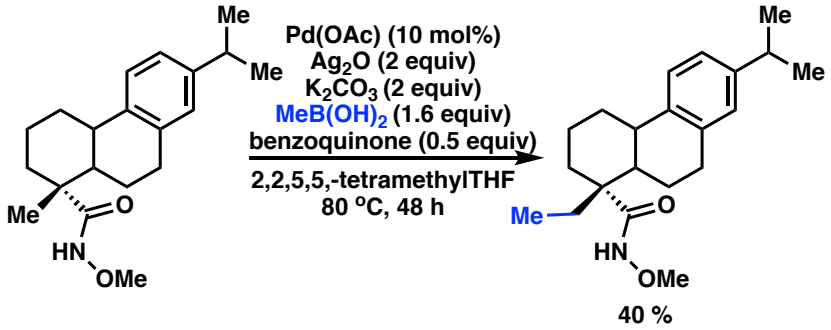
First example of Pd^{II}-catalyzed alkylations with methylboroxine and alkylboronic acids





Ethers, alchols, and esters tolerated
Mono- and di-methylation observed

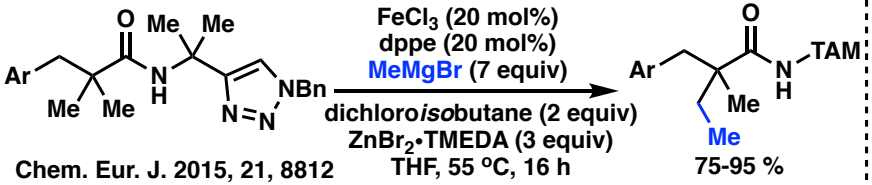
Hydroxamic ester as directing group



Diversification of derivate of dehydroabietic acid (natural product, acts as an efficient BK channel opener)

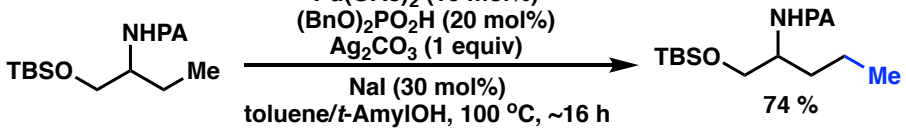
J. Am. Chem. Soc. 2006, 128, 12634
J. Am. Chem. Soc. 2008, 130, 7190

Triazole/Amide-directed Methylation



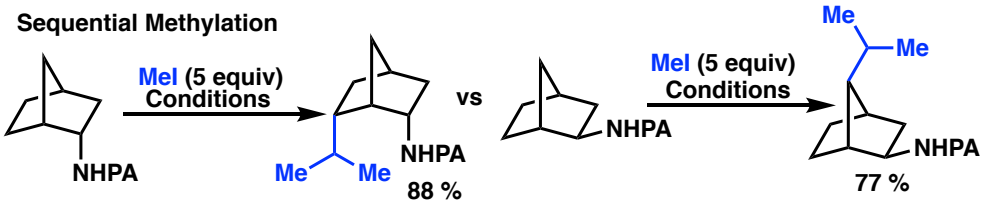
Chem. Eur. J. 2015, 21, 8812

Picolinamide (PA)-directed γ-Methylation



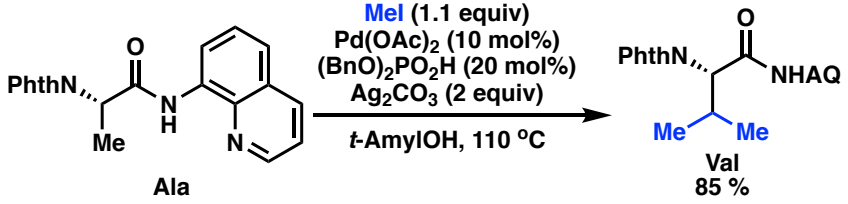
Use of phosphoric acid as phase-transfer catalyst to control [Ag$^+$]
Scope includes cyclic substrates, esters, & ethers, as well as CD_3I and $^{13}CH_3I$

Sequential Methylation



J. Am. Chem. Soc. 2013, 135, 2124

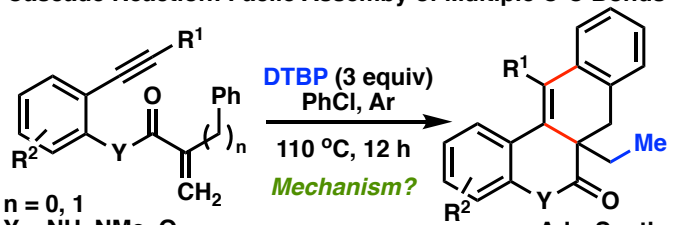
Aminoquinoline (AQ)-directed β-methylation



Applied method to diastereoselective synthetis of β-alkylated amino acids
Labeling with CD_3I and $^{13}CH_3I$

J. Am. Chem. Soc. 2013, 135, 12135

Cascade Reaction: Facile Assemby of Multiple C-C Bonds

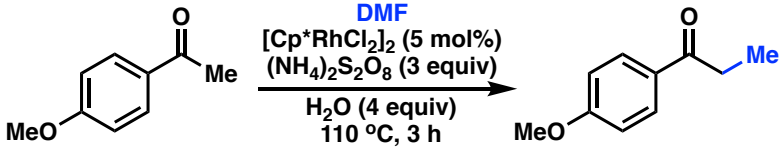


Metal Free Conditions

Adv. Synth. Catal. 2017, 359, 3602

α-Methylation of Aryl Ketones

First example of DMF as a methyl source

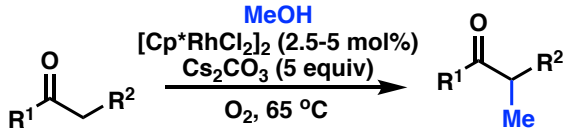


Various substituted arenes and α-substituted ketones tolerated

Org. Lett. 2014, 16, 66

α-Methylation of Ketones

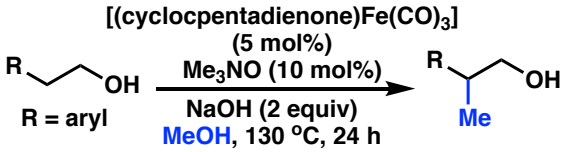
Methanol as methyl source



Angew. Chem. Int. Ed. 2014, 53, 761

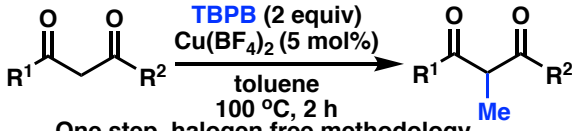
β-Methylation of Alcohols

Bench stable first row transition metal catalysis



ACS Catal. 2019, 9, 8575

α-Methylation of 1,3-Diketones using *tert*-Butyl peroxybenzoate (TBPB)



One step, halogen free methodology

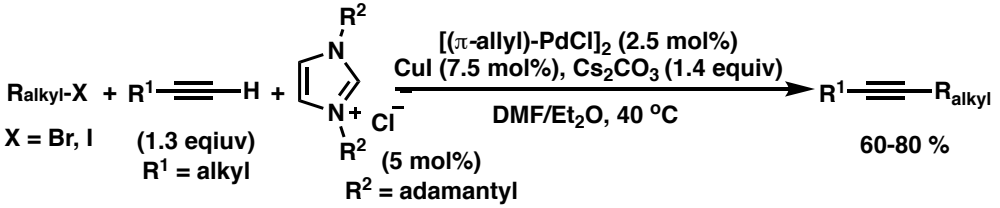
R^1 = aryl, alkyl, alkenyl

R^2 alkyl, alkoxyl, benzyloxy

J. Org. Chem. 2014, 79, 11285

sp Methylation

Methods for alkylation of terminal alkynes are sparse - a few papers address alkylation using carbene ligands but do not include methyl electrophiles in their scope



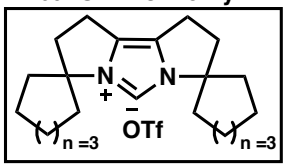
First use of a carbene ligand in a x-coupling with alkyl electrophiles (typical phosphine ligands found to be ineffective)

Functional group tolerance for cyano, halide, alcohol, and alkene groups on alkyl halides

However, limited scope of alkynes - substrate dependent reaction conditions and no methyl halide in scope

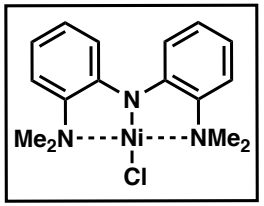
J. Am. Chem. Soc. 2003, 125, 13642

Conditions as above, with inclusion of 2° alkyl electrophiles, but still no methyl



Tetrahedron Lett. 2006, 47, 2925

Nickel Catalysis: First example of primary alkyl chlorides

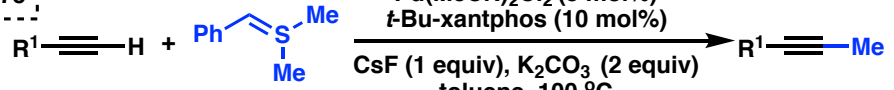


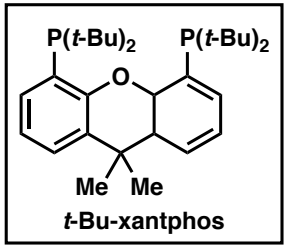
CuI (3 mol%), *n*-BuN_4I (20 mol%), Cs_2CO_3 (1.4 equiv)
dioxane, 140 °C, 16 h

J. Am. Chem. Soc. 2009, 131, 12078

Cross-Coupling using Sulfonium Ylides

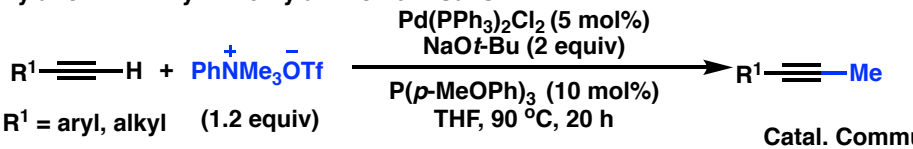
1st example of a methylated alkyne formed from C(sp)-C(sp^3) cross-coupling





J. Org. Chem. 2013, 78, 10421

Methylation with Aryltrimethylammonium Salts

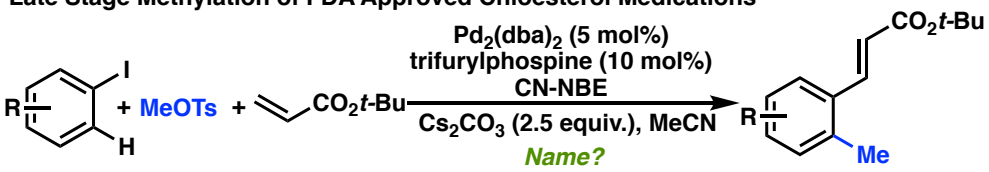


Moderate yields

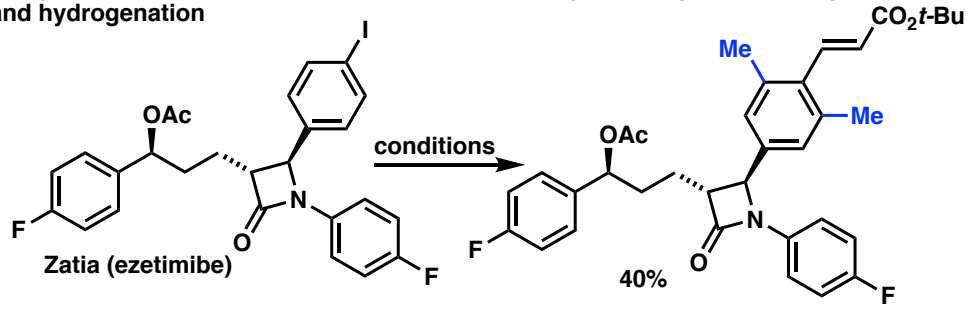
Catal. Commun. 2020, 133, 105835

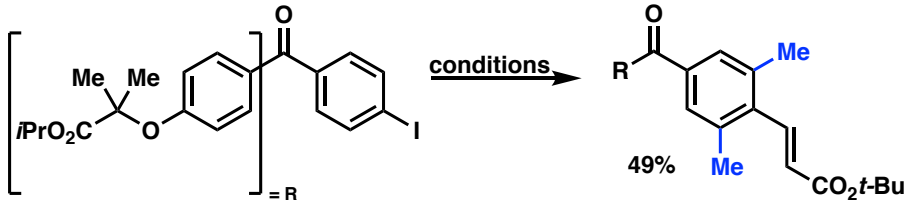
Late Stage Methylation of Pharmaceuticals

Late Stage Methylation of FDA Approved Chloesterol Medications



Six types of ipso terminations: Heck, Suzuki, Sonogashira, cyanation, borylation, and hydrogenation

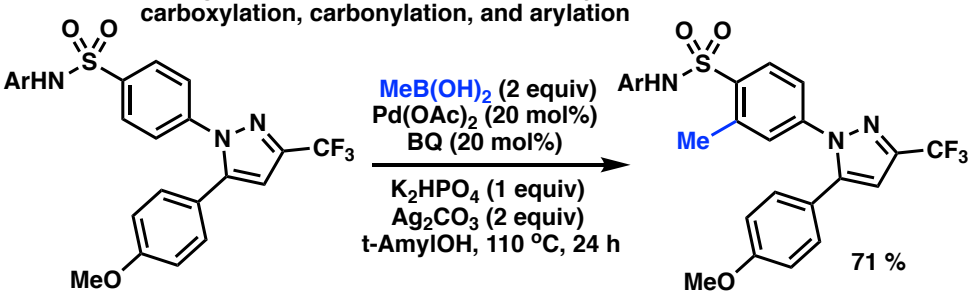




J. Am. Chem. Soc. 2019, 141, 15986

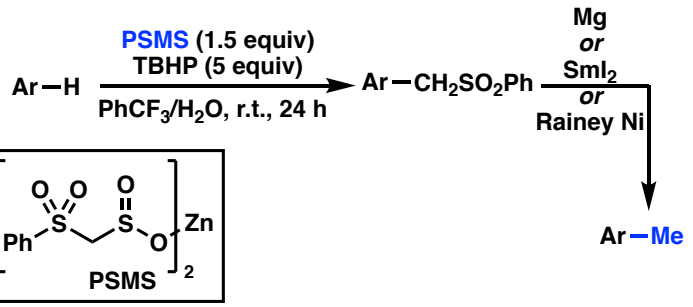
Sulfonamide Pharmacophores as Directing Group

Divergent C-H functionalizations: methylation, olefination, iodination, carboxylation, carbonylation, and arylation

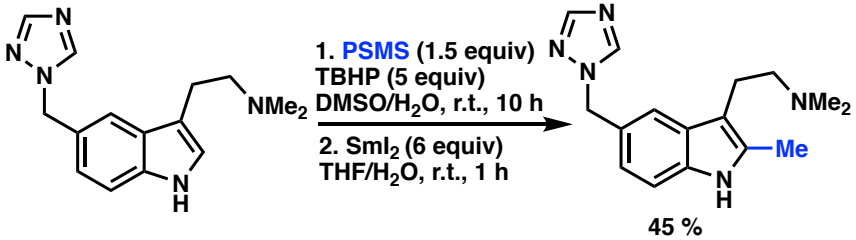


Analog of Celebrex (celecoxib)
NSAID

Acc. Chem. Res. 2012, 45, 6, 788
J. Am. Chem. Soc. 2011, 133, 7222



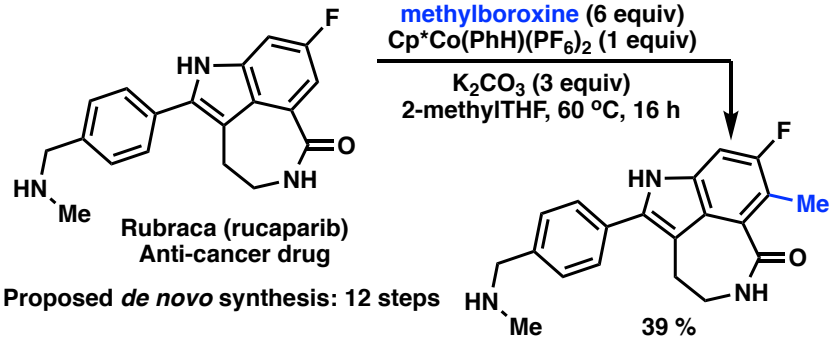
-> Easy separation of product from starting material
-> Tolerates free N-H and O-H bonds
-> Slow reactivity with electron deficient substrates



Maxalt (rizatriptan)
Treats migraines

J. Am. Chem. Soc. 2014, 136, 4853

Methylboroxine Methylation



Rubraca (rucaparib)
Anti-cancer drug

Proposed *de novo* synthesis: 12 steps

Nat. Chem. 2020, 12, 511