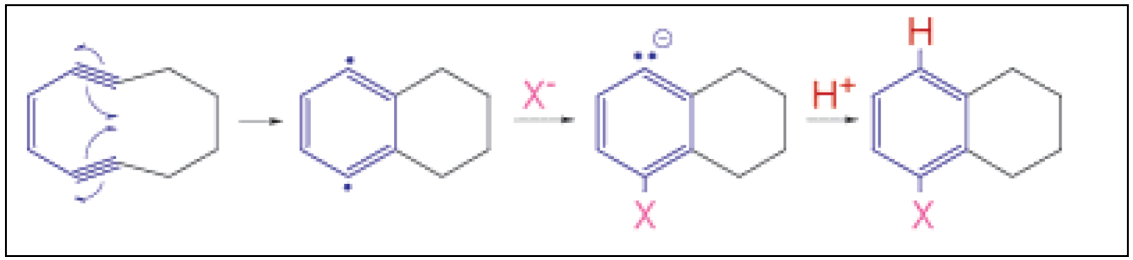
Nucleophilic Addition to a *p*-Benzyne Derived from an Enediyne: A New Mechanism for Halide Incorporation into Biomolecules

Perrin, C. L.; Rodgers, B. L.; O'Connor, J. M.
J. Am. Chem. Soc. **2007**, *ASAP*

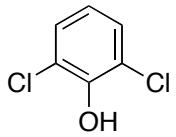


John Maciejewski

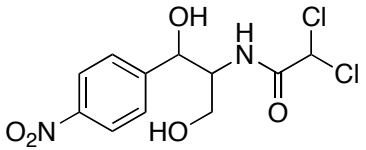
Current Literature 4/7/07

Halogens in Natural Products

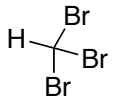
- To date ~4,500 know halogenated natural products
- Halogen incorporation changes physical properties
  - binding affinity and selectivity
- Up to 20% pharmaceuticals on market are halogenated
- Most pharmaceuticals contain fluorine or chlorine where most natural products contain bromine or iodine



2,6-Dichlorophenol
sex-pheromone of lone star tick



Chloramphenicol
antibiotic activity

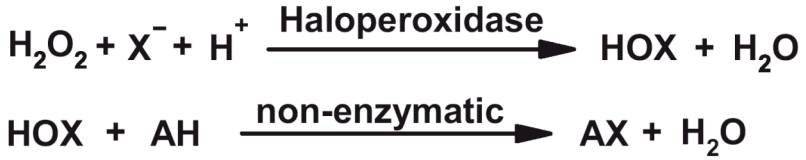


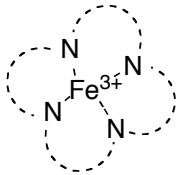
Bromoform
marine algae

Yarnell, A. *Chem. Eng. News* **2006**, *84*, (21), 12
Murphy, C. D. *J. Appl. Microbiol*. **2003**, *94*, 539

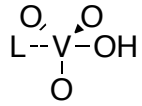
Haloperoxidases

- Most halogenase enzymes oxidize halide ions to *electrophilic* species or radical species that react with the target substrate
- Haloperoxidases may use either heme-iron or vanadium cofactors with enzyme to generate hypohalous acid





Heme-iron cofactor



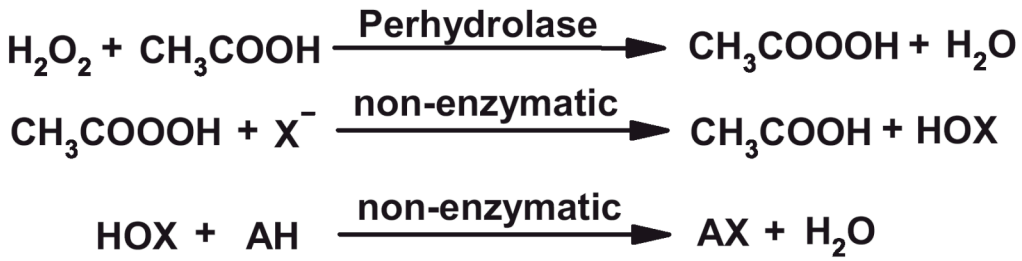
Vanadium cofactor

Yarnell, A. *Chem. Eng. News* **2006**, *84*, (21), 12
van Pee, K.-H.; Patallo, E. P. *Appl. Microbiol. Biotechnol*. **2006**, *70*, 631
Murphy, C. D. *J. Appl. Microbiol*. **2003**, *94*, 53

Perhydrolases

- Perhydrolases catalyze formation of short-chain aliphatic peracids

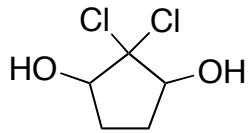
- Peracids then oxidize halide ions to form hypohalous acids



van Pee, K.-H.; Patallo, E. P. *Appl. Microbiol. Biotechnol*. **2006**, *70*, 631

First Discovered Haloperoxidase

- First observed by Shaw (1959) while studying biosynthesis of caldariomycin
- Occurs in fungus *Caldariomyces fumago*
- Chloroperoxidase from *C. fumago* most widely studied halogenase (~42kDa)
- Uses heme-iron as oxidizing cofactor for activation of C-H bonds



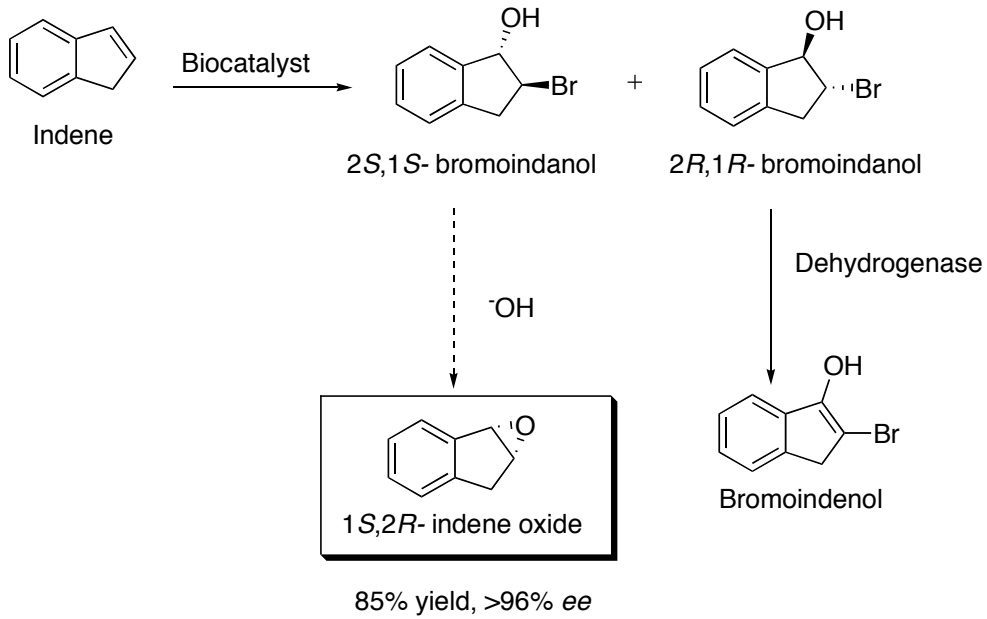
Caldariomycin

Sundaramoorthy, M.; Terner, J.; Poulos, T. L. *Chem. Bio*. **1998**, *5*, 461
Shaw, P. D.; Hager, L. P. *J. Biol. Chem.* **1959**, *234*, 2565

Haloperoxidases as Biocatalysts

- Merck used haloperoxidase to synthesize starting materials for HIV-1 protease inhibitor

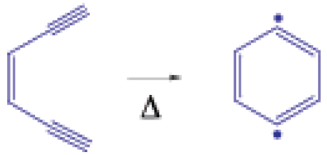
- Biocatalyst used on process scale to produce several kilograms of optically active material



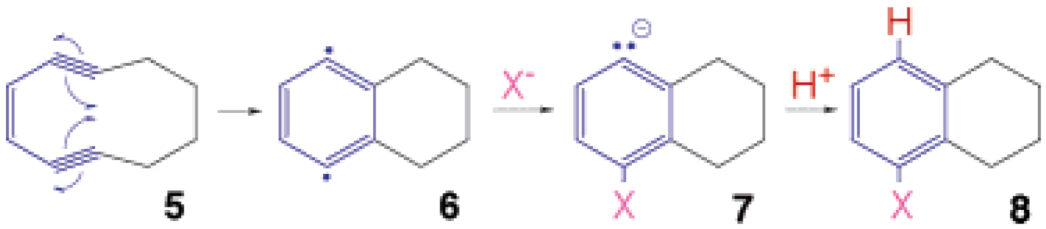
Zhang, J.; Roberge, C.; Reddy, J.; Connors, N.; Chartrain, M, Buckland, B.; Greasham, R. *Enzyme Microb. Technol*. **1999**, *24*, 86

Halogen Incorporation via *p*-Benzyne Pathway

Bergman cyclization

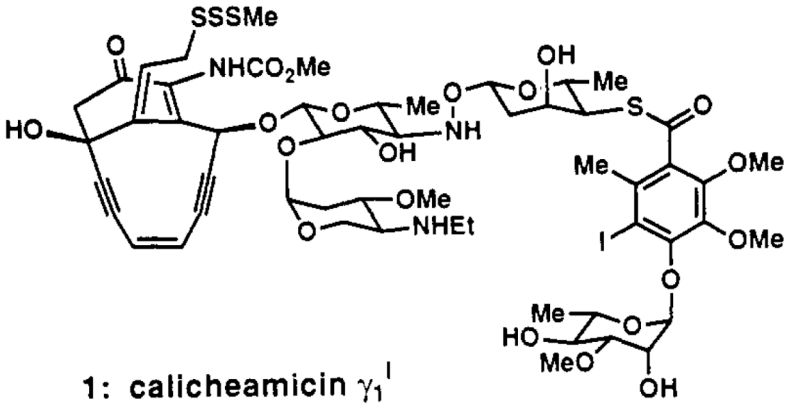


Bergman, R. G. *Acc. Chem. Res*. **1973**, *6*, 25

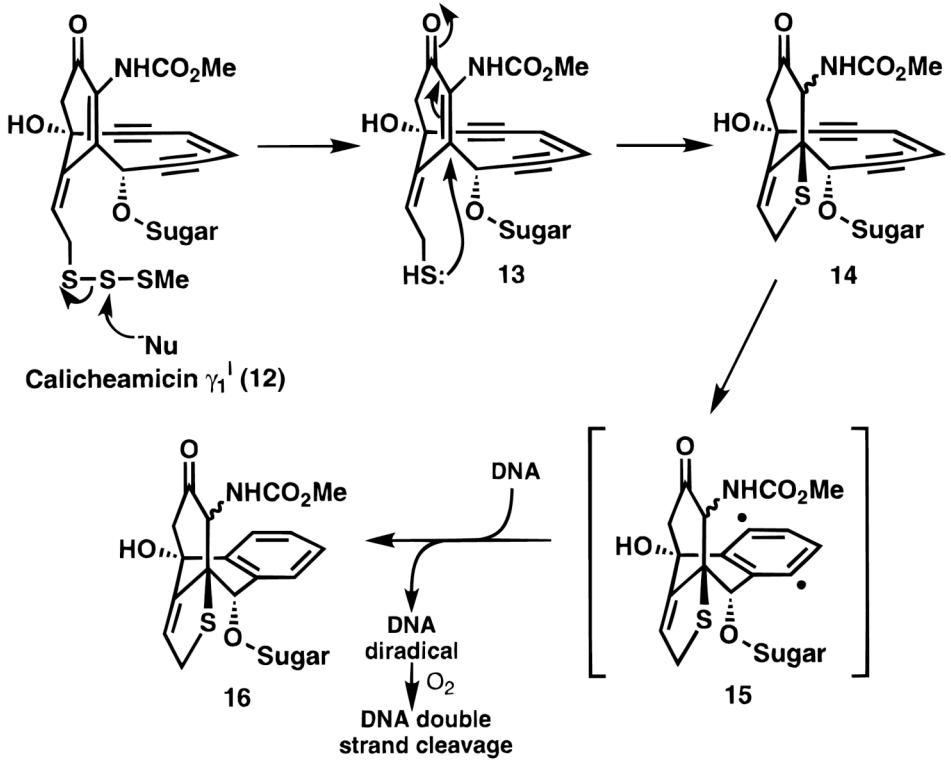


Sequence incorporating an enediyne cyclization (**5**), *nucleophilic* attack of halide onto *p*-benzyne (**6**), then subsequent protonation of sp^2 arene (**7**) to form **8**

Enediyne Scaffolds in Natural Products



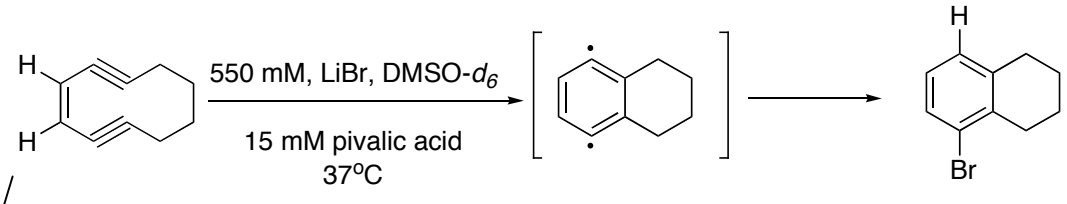
1: calicheamicin γ_1^I

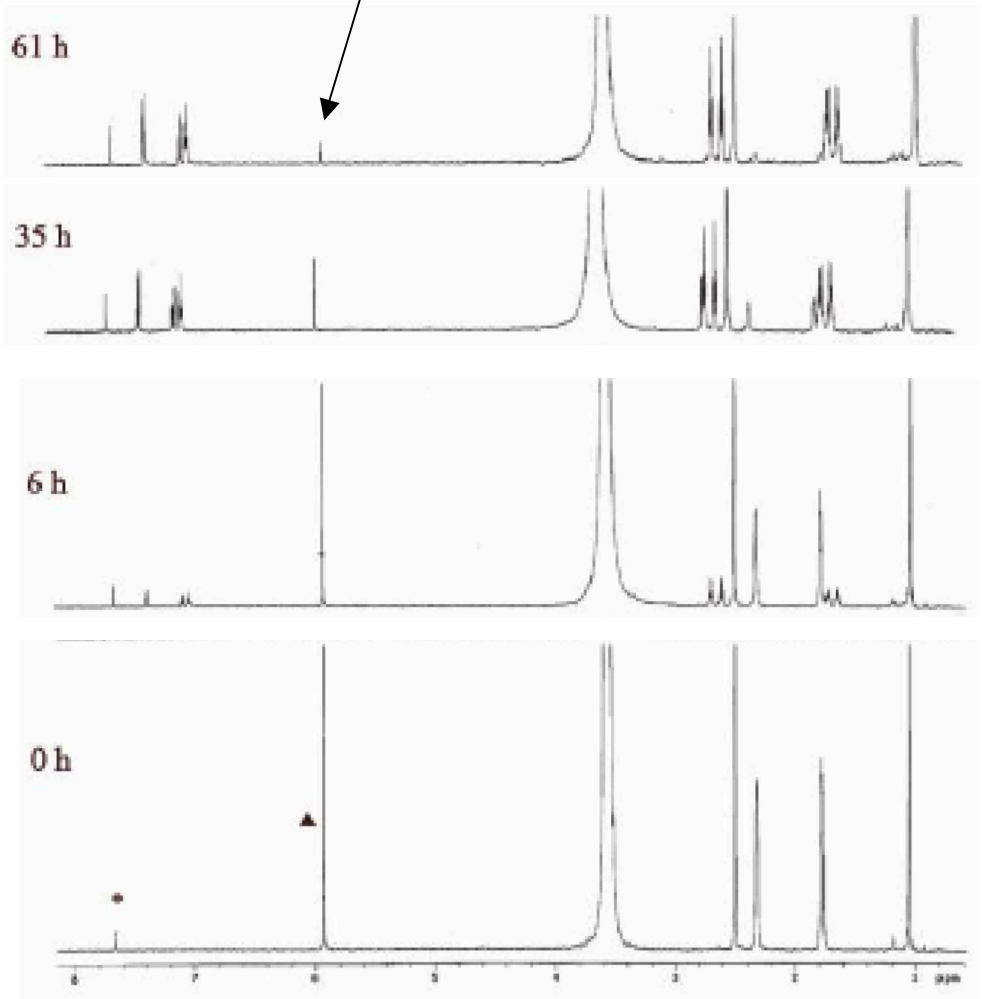


- Natural products calicheamicin and esperamicin contain enediyne trigger
- Abstracts hydrogen atoms from sugar phosphate backbone of DNA

Smith, A. L.; Nicolaou, K. C. *J. Med. Chem.* **1996**, *39*, 2103
Nicolaou, K. C.; Zuccarello, G.; Riemer, C.; Estevez, V. A.; Dai, W.-M. *J. Am. Chem. Soc.* **1992**, *114*, 7360

Results of Nucleophilic Attack on Enediyne



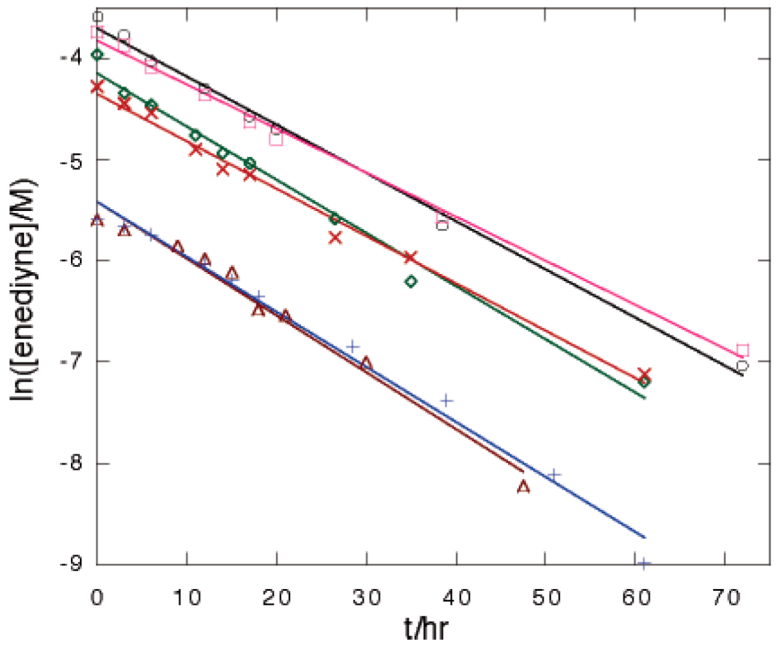


Selected ^{1}H NMR from supporting info.

Reaction monitored by disappearance of olefin peak with respect to internal standard (1,3,5-trimethylbenzene)

Yields >90%

Kinetics of Cyclization



ln[enediyne] vs. t/hr of indicated entries

MX	$[5]_0$/mM	$[X^-]$/mM	[HA]/mM	$10^5 k/s^{-1}$	%yield
LiI	75	750	90	1.42	100
LiI[a]	4	550	20	1.38	100
LiI	4	55	20	1.31	100
LiI	75	370	90	1.35	98
LiI[b]	4	550	20	1.23	55
LiBr	3.8	550	15	1.51	100
LiBr	19	576	20	1.46	100
LiBr[a]	3.8	550	15	1.56	92
LiBr[a]	14	584	20	1.30	92
LiBr	24	360	190	1.21	77
LiBr	28	420	84	1.32	71
LiCl	3.8	550	15	1.30	99
LiCl[a]	3.8	550	15	1.59	37
none	15.5	0	0	2.07	0

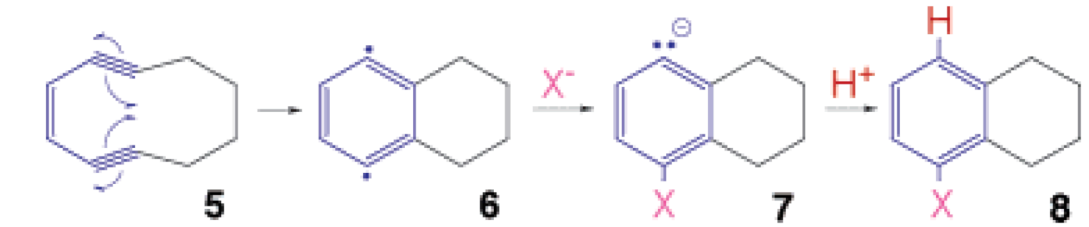
[a] + 20% D_2O. [b] + 50% D_2O.

$$-\text{d[enediyne]}/dt = k\text{[enediyne]}$$

$$k_{avg} = 1.38 \times 10^{-5}\ s^{-1}$$

Rate independent of conc. of acid, halide, or halide used

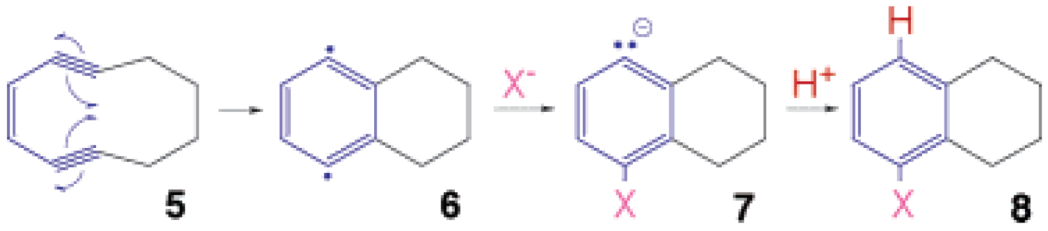
Proposed Formation of **8**



- Formation of weak σ-bond through σ*-orbital in **12**

- Nucleophilic addition to **12** results into **7** which is then protonated by a proton source (pivalic acid).

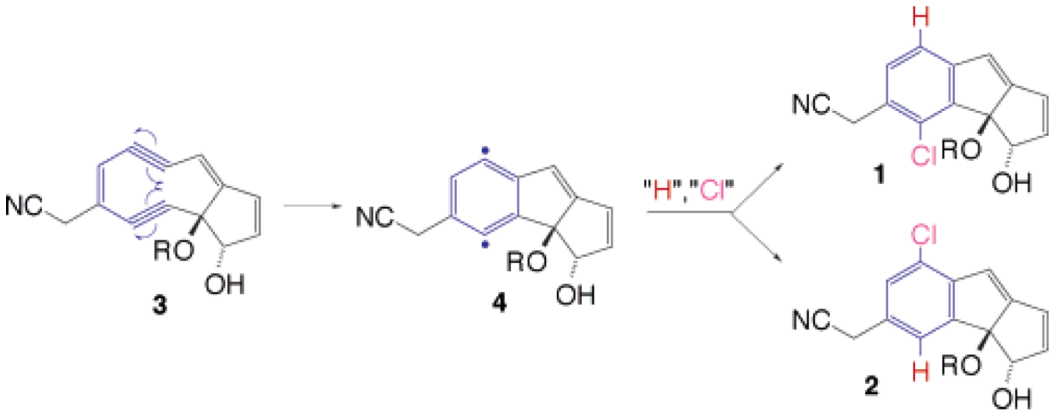
Deuterium Incorporation



- Product **8** was deuterium enriched by (X = Cl, Br, I) 67%, 51%, 42%, respectively, by ^{1}H NMR, including when no D_2O added.

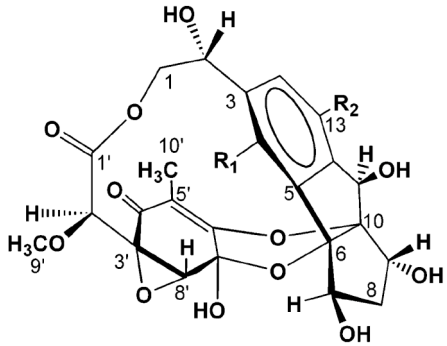
- Deuterium abstraction from DMSO-d_6 (in presence of pivalic acid) supports strong base generated in reaction (**7**).

Is This an Active Pathway in Nature?



Proposed (partial) mechanism for synthesis of Cyanosporasides **1** and **2**

Asymmetric addition to *p*-benzynes shows little selectivity and may explain the 1:1 (isolated) ratio of Cyanosporasides **1** and **2** (above), along with Sporolides **A** and **B** (below)



A R^1 = Cl R^2 = H
B R^1 = H R^2 = Cl

Sporolides **A** and **B**

Buchanan, G. O.; Williams, P. G.; Feling, R. H.; Kauffman, C. A.; Jensen, P. R.; Fenical, W. *Org. Lett.* **2005**, *7*, 2731

Conclusion

- A new mechanism whereby halogenation of natural products occurs through *nucleophilic* halogen attack onto the activated substrate has been discussed

- This process may be able to explain the biosynthesis of sporolides and cyanosporasides

- The kinetic data is consistent with a first-order process dependent only on the enediyne

- Experiments are underway to investigate the scope and utility of incorporating nucleophiles onto aromatic systems through this process