

No Analytical crib available
December 13, 2008
Written by Professor Ramachandran

No Inorganic crib available
December 13, 2008
Written by Professor Robinson

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December 13, 2008
Written by Professor Dian

December 13, 2008

CRIB

Biochemistry Cumulative Examination

5 questions 20 points each

Techniques in Molecular Biology

1. (a) What is a DNA chip (a DNA microarray)? Describe in some detail (but ≤ 1 pg in blue book).

Typically an array of immobilized SS oligonucleotides. Can represent almost anything...entire genome...expressed genes...see below....Is probed with denatured (ssDNA), look for hybridization signal, from fluorescently labeled samples.

How might it be used to

(b) Assess changes in gene expression levels between normal cells and cancer cells?

Array should contain short oligonucleotides from as many expressed genes as possible. Isolate mRNA, make cDNA, label "normal" cDNA with green fluorescence, "cancer" cDNA with red fluorescence, probe single chip with mixture of cDNAs and analyze at both wavelengths of light.

(c) Identify (most or) all of the species of bacteria present in an infection

Array should contain oligos from many species corresponding to regions that vary significantly between microorganisms.

(d) Screen for a genetic predisposition to breast cancer (mutation in the BCRA gene, where a point mutation almost anywhere in the gene is associated with cancer).

Array should contain short oligos that cover entire length of wild type BCRA gene. As control, need denatured DNA (blood samples) from normal individuals. Denature the DNA in the samples - a process that separates the two complementary strands of DNA into single-stranded molecules. Cut the long strands of DNA into smaller, more manageable fragments. and Label each fragment by attaching a fluorescent dye. The individual's DNA is labeled with green dye and the control - or normal - DNA is labeled with red dye. Both sets of labeled DNA are then inserted into the chip and allowed to hybridize - or bind - to the synthetic BRCA1 DNA on the chip. If the individual does not have a mutation for the gene, both the red and green samples will bind to the sequences on the chip. Wild type will bind preferentially if the patient has a mutation in a given region of the gene.

(e) Screen for alternative splicing of mRNAs

Array should contain oligos specific to all possible products of alternative splicing

Be sure to include in your discussion what type of controls are run to correctly interpret data.

2. You have discovered a very important protein that, when present, causes cancer cells to grow and divide at 20 times the normal rate. Further study reveals that the protein is regulated by the retinoic acid receptor (RXR), which binds DNA upstream of the gene encoding this protein and acts as a transcriptional regulator. You also discover that a derivative of retinoic acid slows tumor growth (\$\$\$). Your drug candidate (retino-125) binds to the RXR protein and induces a conformational change that...

1. may alter the ability of RXR to bind to DNA with the same affinity as the RXR:retinoic acid complex. Alternatively it... 2. may still bind with similar affinity but not allow transcriptional activation. Outline strategies to test these two hypotheses.

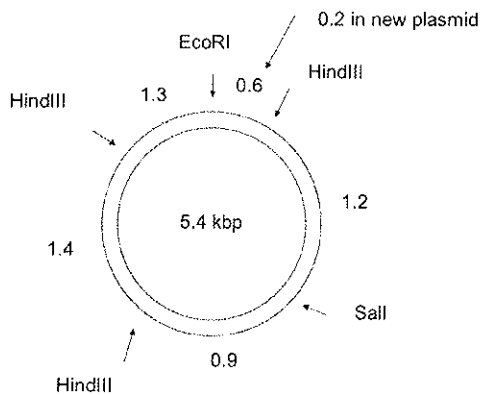
1. A DNA-gel-shift assay can assess the effect of drug on protein-DNA binding interaction

2. Transcription is assayed using a reporter gene such as *lacZ* in a transcriptional fusion using the promoter region upstream of the gene encoding your protein. Could also look at mRNA levels through hybridization.

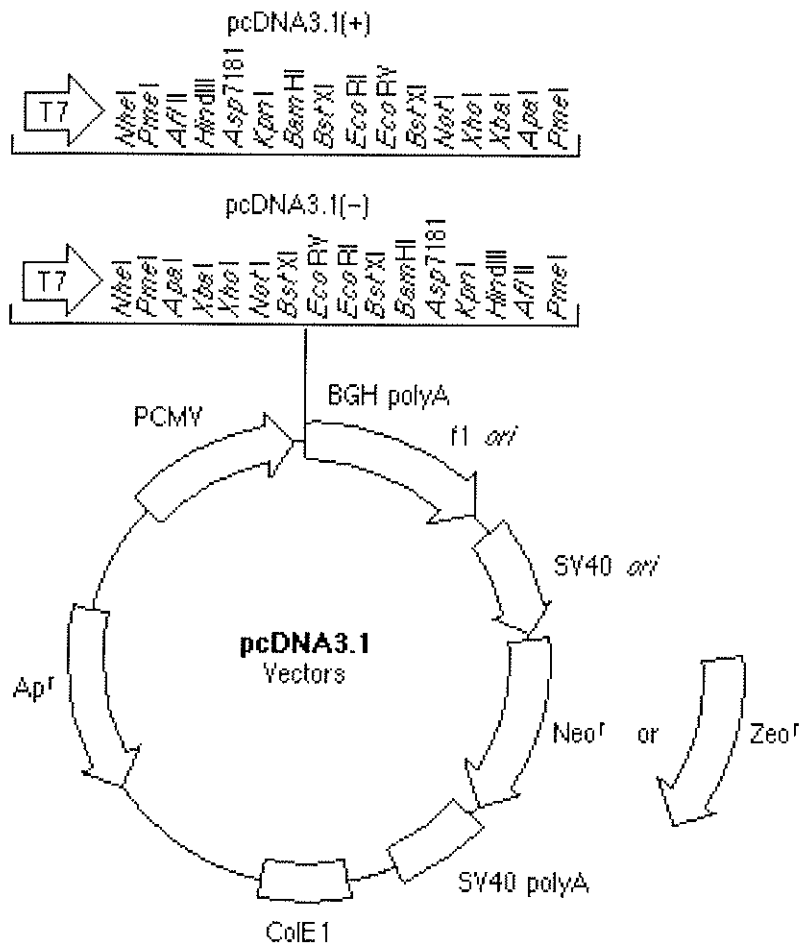
3. Most site-directed mutagenesis kits use polymerases that replicate the entire plasmid using mutagenic primers. After doing a reaction, you note that your protein is no longer expressed from the mutated plasmid and begin your trouble-shooting by doing a restriction digest of the plasmids with the enzyme EcoRI, which cuts just once. While the fragment size of the starting plasmid was 5.4 kb, as expected, the new plasmid is smaller, only 5.0 kb. Next, you do a more in depth series of restriction digests (single and double digests), of both the original plasmid and the new plasmid, to construct a restriction map and see how they differ. The following data is obtained:

Enzyme	Original plasmid Fragment sizes (kb)	New plasmid Fragment sizes (kb)
EcoRI	5.4	5.0
HindIII	2.1, 1.9, 1.4	2.1, 1.5, 1.4
Sal I	5.4	5.0
EcoRI and HindIII	2.1, 1.4, 1.3, 0.6	2.1, 1.4, 1.3, 0.2
Sal I and HindIII	1.9, 1.4, 1.2, 0.9	1.5, 1.4, 1.2, 0.9

Draw circular restriction maps for both plasmids based on this data. (Don't worry about drawing to scale just label distance between sites, as shown in example of restriction map below). Where is the difference between them? One possible solution is shown below. The EcoRI to HindIII fragment decreased from 0.6 to 0.2 kbp in length.



4. Identify each of the elements labeled in the plasmid below and explain the *purpose* for their presence on a cloning vector. Based on the elements that are present, what could this plasmid be used for?



PCMV: cytomegalovirus promoter: eukaryotic expression
 T7: bacterial phage promoter followed by multiple cloning site
 BGH polyA: polyadenylation signal for inserted gene: role in nuclear export, translation and/or stability of mRNA
 fl ori: preparation of single-stranded DNA by phage infection
 Neomycin resistance: selection for plasmid in eukaryotic cells
 SV40 ori: replication in eukaryotic cells
 SV40 polyA: polyadenylation signal for neomycin resistance gene
 ColE1: Origin of replication in E. coli
 Apr: Ampicillin resistance, selection for plasmid in bacteria

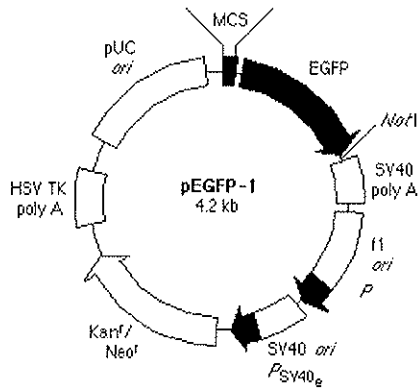
This plasmid is used as “shuttle vector” that can propagate in two different host species.

5. The following vector is designed to simplify the process of making a fusion between a protein of interest and an engineered green fluorescent protein (EGFP). Design two PCR primers to amplify the gene shown below and clone it into the multiple cloning site (MCS) of the vector, in order to generate a protein fusion. STRATEGY: Incorporate restriction sites into your primers so that you can amplify the gene and clone it into the vector using restriction enzyme digestion and ligation reactions. For the purpose of this exercise, we will assume that none of the enzymes listed in the MCS cut within your gene. (Recognition sites for enzymes are underlined...all are 6 bp long).

Your grade will reflect both your ability to design PCR primers correctly (indicate 5' end!), and the success of your strategy to make the fusion protein.

MCS

TA GCG CTA CCG GAC TCA GAT CTC GAG CTC AAG CTT CGA ATT CTG CAG TCG ACG GTA CCG CCG GCC CGG GAT CCA CCG GTC GCC ACC ATG GTG → EGFP
 Eco47III BglII XhoI SacI HindIII EcoRI PstI SmaI KpnI SacII ApaI XmaI BamHI AgeI
 Eco136II AclI Asp718I Esp120I SmaI



Many solutions, however...

- primers are designed to amplify gene and clone into MCS
- must anneal to 5' or 3' end of gene for amplification
- primers must also contain restriction site for cloning, added as extension to 5' end of primer
- must remove termination codon at 3' end of gene
- must be in frame with EGFP at 3' end

Primers highlighted in yellow.

5'-GGGAGATCTATG GCG AGC GTA CAG CTG
 BglIII M A S ... (Anneals to beginning of gene and adds BglIII restriction site)

5'-AAG GAG CCG GGC GTT ACC ACC GGT CGG ACC ATG GTG
 K E P G V T T G R T M V (end of gene fused to Met of EGFP)

3'-TTC CTC GGC CCG CAA TGG TGG CCA GCC - 5'

Following amplification of gene, PCR product (and pEGFP-1 vector) are cut with BglIII and AgeI and gene is ligated into multiple cloning site.

1/1
 ATG GCG AGC GTA CAG CTG
 M A S V Q L
 91/31
 TTC GTG GTG TTT GTC GGA
 F V V F V G
 181/61
 TTC ATC GGT GAG AAR CCG
 F I G E K R
 271/91
 TCA GTA GCA GAA AAC ATG
 S V A E N H
 361/121
 TCC GGT GGT CAG CGT CAG
 S G G Q R Q
 451/151
 GAT GGT GCA CTG COT GTG
 D A R L R V
 541/181
 GTC GAA GCG ATG ACG CTG
 V E A H Y L
 631/211
 CCG GCA GAC CGT TTT GTC
 P A D R F V
 721/241
 CAG GTG GAG CTG CCG ATG
 Q V E L P R
 811/271
 ATT CCG CCG GAA CAT CTA
 I R P E H L L
 901/301
 CAA ATC CAT ATC CAG ATC CCT TCC ATT CGT
 Q I H I Q I P S I R
 931/311
 CAA AAC CTG GTG TAC CCG CAG AAC GAC GTG
 Q A H L V Y R Q C H D V
 961/321
 GTG TTG GTA GAA GAA GGT GCC ACR TTC GCT
 V L V E G A T F H
 991/331
 ATC GGC CTG CCG CCA GAG CGT TGC CAT CTG
 I G L P P E R C H L F
 1021/341
 TTC CGT GAG GAT GGC ACT GCA TGT CGT CGA
 F R E D G T A C R R L
 1051/351
 CTG CAT AAG GAG CCG GGC GTT TAA
 L H K E P G V *

All of the questions are from the following article (*JACS*, 2008, 130, 14891) by Professor Michael Krische, who discussed this work during the Negishi-Brown Symposium in October.

J|A|C|S

ARTICLES

Published on Web 10/06/2008

Enantioselective Iridium-Catalyzed Carbonyl Allylation from the Alcohol or Aldehyde Oxidation Level via Transfer Hydrogenative Coupling of Allyl Acetate: Departure from Chirally Modified Allyl Metal Reagents in Carbonyl Addition

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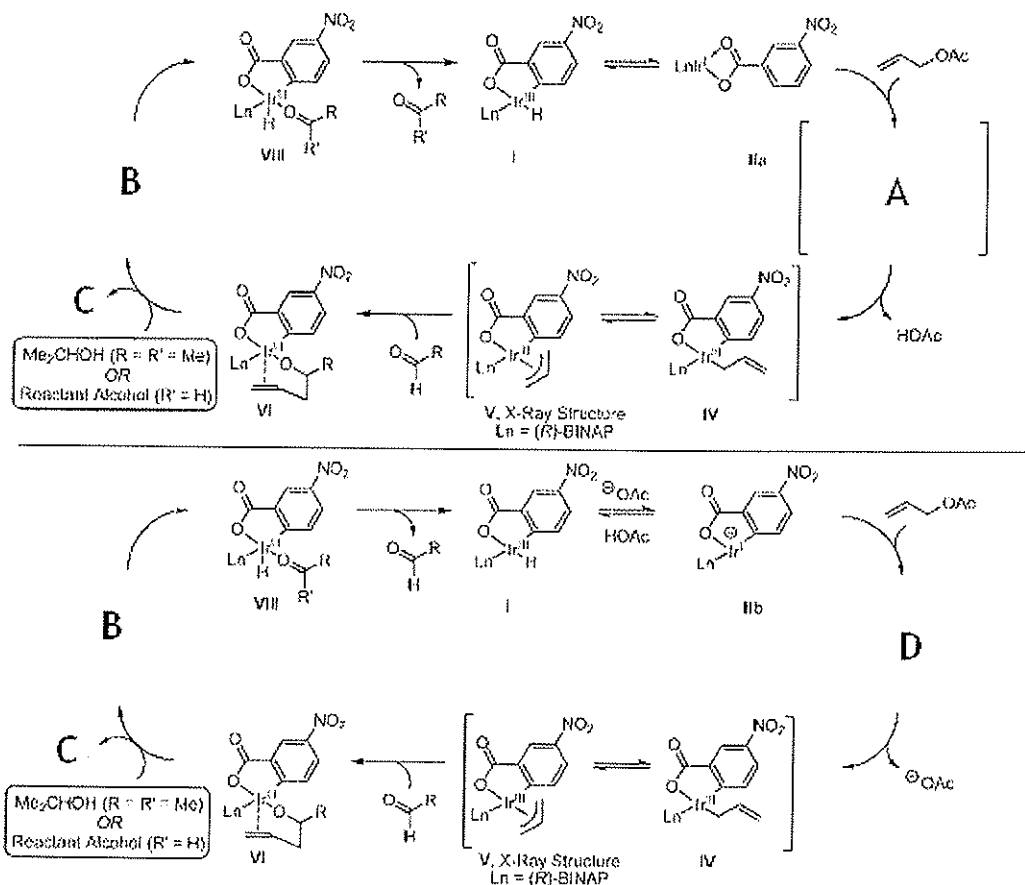
Received July 22, 2008; E-mail: mkrische@mail.utexas.edu

I. Provide the missing structures (A-D) in the proposed mechanism of the transfer hydrogenative coupling (30 points).

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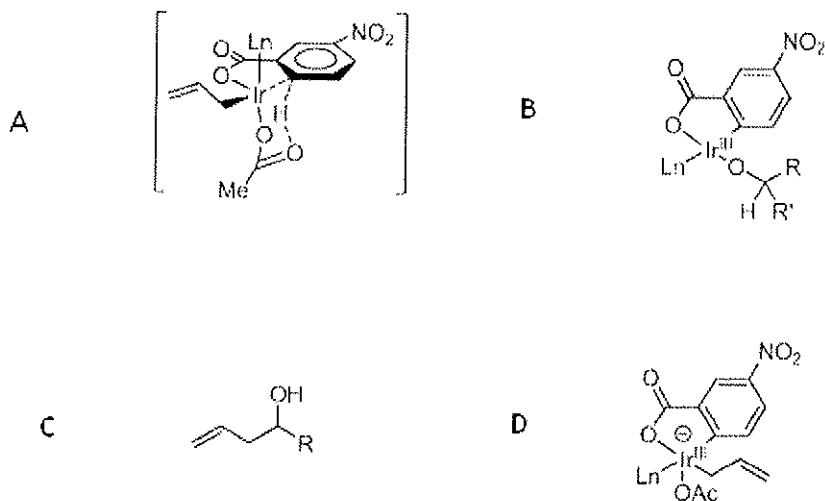
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Scheme 4. Postulated Catalytic Mechanisms for the Iridium-Catalyzed Transfer Hydrogenative Coupling from the Alcohol or Aldehyde Oxidation Level (Ln = Chelating Triaryl Phosphino, e.g., (R)-BINAP or (R)-Cl₂MeO-BIPHEP)

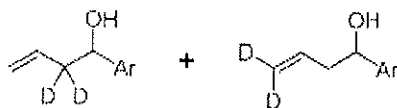
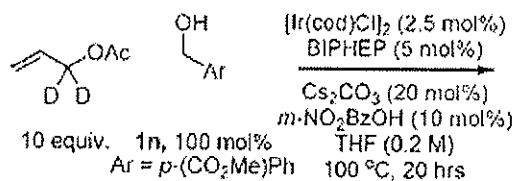


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II. Provide the structure(s) of the expected product(s) from the following reaction (20 pts.)



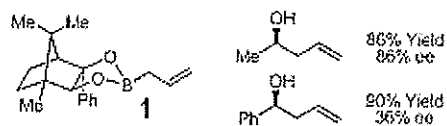
III. Professor Krische has compared his reaction with the following reagents/reactions, which bears the name of the organic chemist who discovered them. (a) Provide the names of five of these chemists for the appropriate reagent/reaction (10 pts). (b) What is BINOL in reaction 9 (5 pts)? (c) Provide the configuration of reagent 4. Explain (5 pts). (d) Write a plausible general mechanism for reaction 9 (30 pts).

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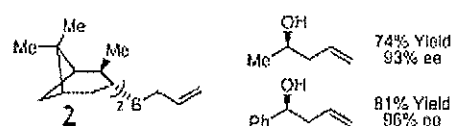
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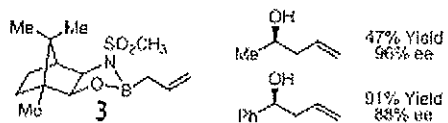
Angew. Chem., Int. Ed. Engl. 1978, 768



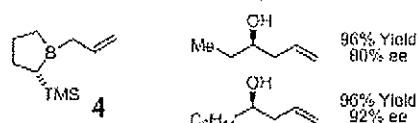
J. Am. Chem. Soc. 1983, 2092



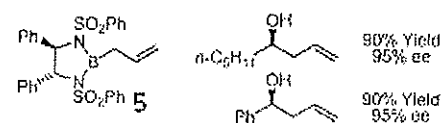
Pure Appl. Chem. 1988, 1607



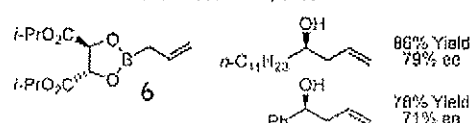
J. Am. Chem. Soc. 1989, 1892



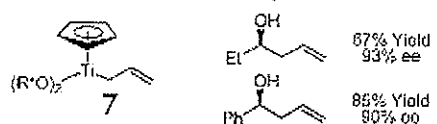
J. Am. Chem. Soc. 1989, 5495



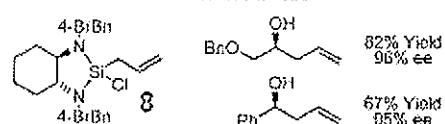
J. Am. Chem. Soc. 1985, 8186



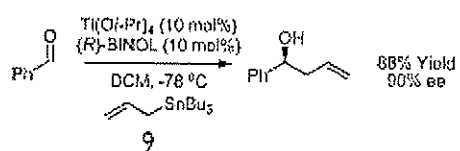
Angew. Chem., Int. Ed. Engl. 1989, 494



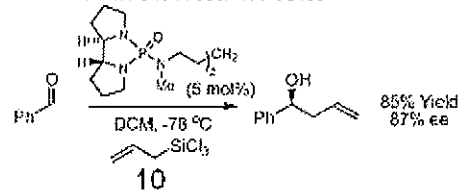
J. Am. Chem. Soc. 2002, 7920



J. Am. Chem. Soc. 1993, 7001
J. Am. Chem. Soc. 1993, 9467

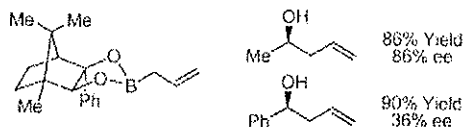


J. Am. Chem. Soc. 2001, 9480

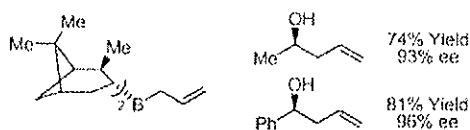


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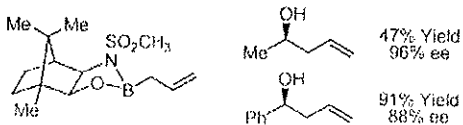
Hoffmann, *Angew. Chem., Int. Ed. Engl.* **1978**, 768



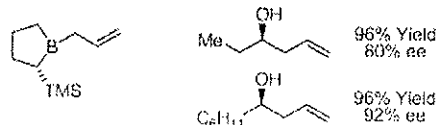
Brown, *J. Am. Chem. Soc.* **1983**, 2092



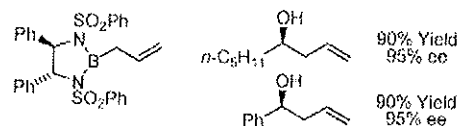
Reetz, *Pure Appl. Chem.* **1988**, 1607



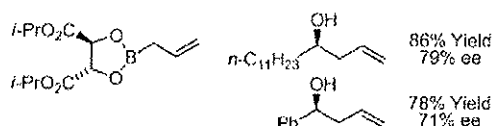
Masamune, *J. Am. Chem. Soc.* **1989**, 1892



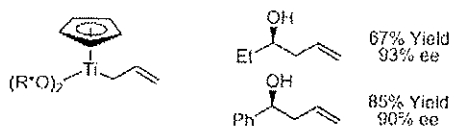
Corey, *J. Am. Chem. Soc.* **1989**, 5495



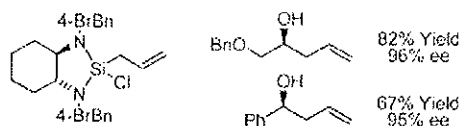
Roush, *J. Am. Chem. Soc.* **1985**, 8185



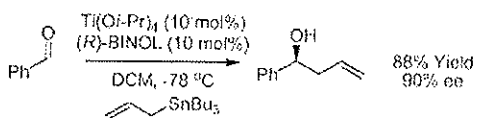
Duhalcor, *Angew. Chem., Int. Ed. Engl.* **1989**, 494



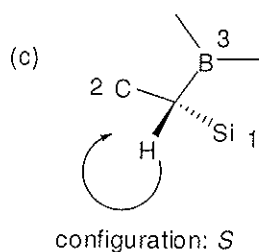
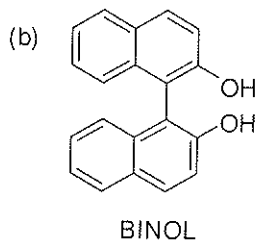
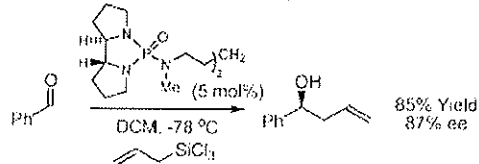
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Denmark *J. Am. Chem. Soc.* **2001**, 9488



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