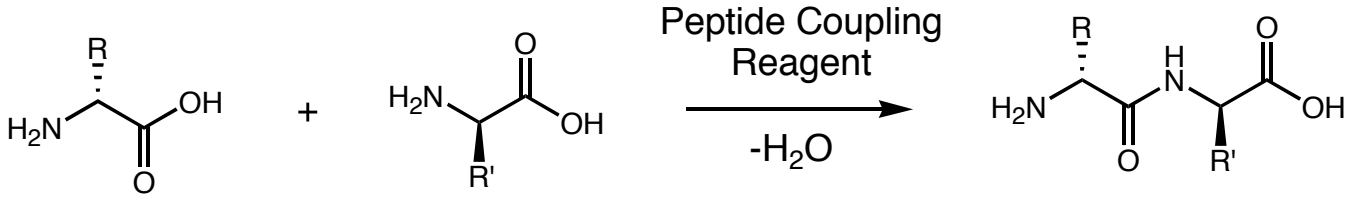
Strategies for Peptide Synthesis: An Overview



Han, S., Kim, Y. *Tetrahedron*, **2004,** *60,* 2447-2467
Albericio, F. *Current Opinion in Chemical Biology*, **2004**, *8*, 211-221
Humphrey, J., Chamberlin, R. *Chem. Rev.*, **1997**, *97*, 2243-2266

Outline

1) Introduction
- General Strategy

2) Coupling reagents
- Carbodiimides
- Uronium Reagents
- Phosphonium Reagents
- Organophosphorous Reagents
- Acid Halogenating reagents
- Racemization Pathways
- Racemization Suppressants

3) Difficult Couplings
- α,α-disubstituted Amino Acids
- Peptide Macrocylizations
- N-methyl Amino Acids
- Segment Condensations

4) Applications
- Process Scale Solid Phase Peptide Synthesis

Introduction

- Amide Bonds are Ubiquitous in Nature

- A large number of Natural products are based upon a peptide framework and exhibit a spectrum of biological activity

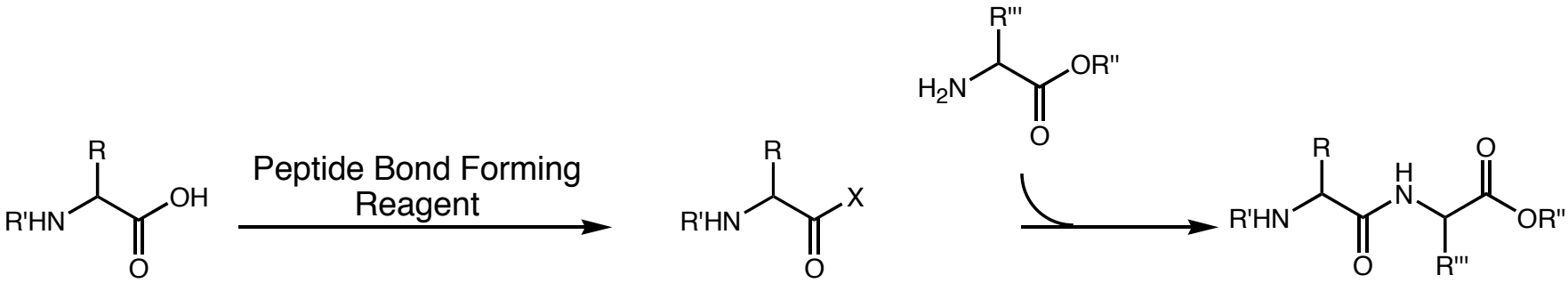
- Currently there are many peptide therapeutics in development

- The current pursuit of non-natural amino acid mimics makes coupling chemistry paramount for drug discovery and scientific advancement

-There is no single strategy for amide bond formation that is a magic bullet

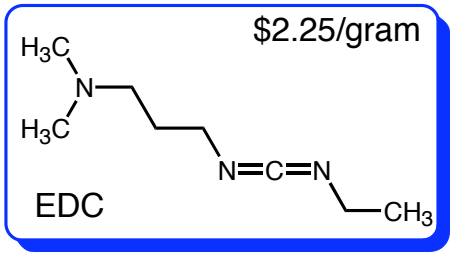
-Advances in coupling chemistry have made formation of the most difficult amide bonds possible

General Strategy For Peptide Bond Formation

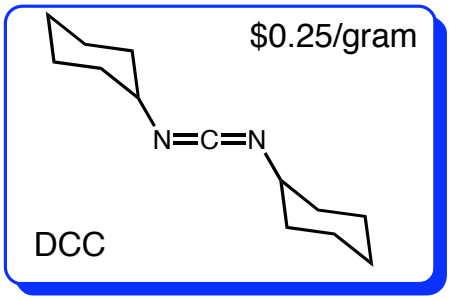


X = Activating Group

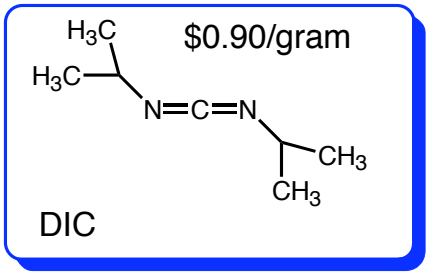
Carbodiimides: Representative Examples/ Comparisons



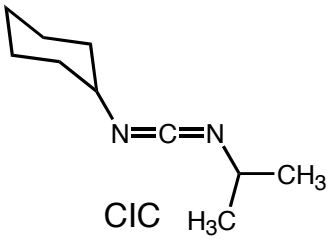
Water Soluble by-product is easily removed in aqueous work-up

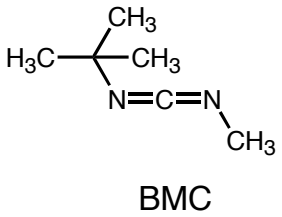


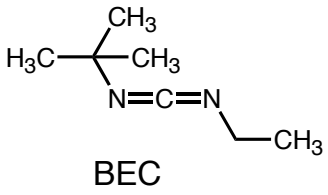
Urea formed is partially soluble in many solvents and hard to purify via column chromatography

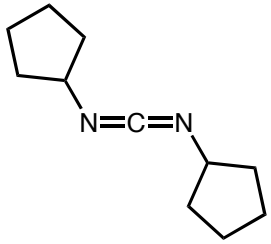


Urea formed is soluble in most organics. This is advantageous in solid phase synthesis.

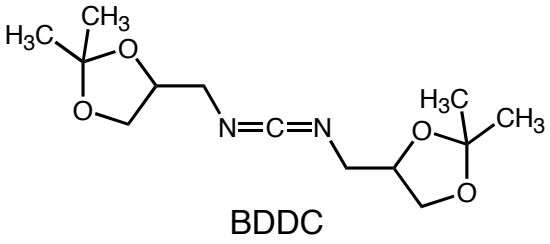




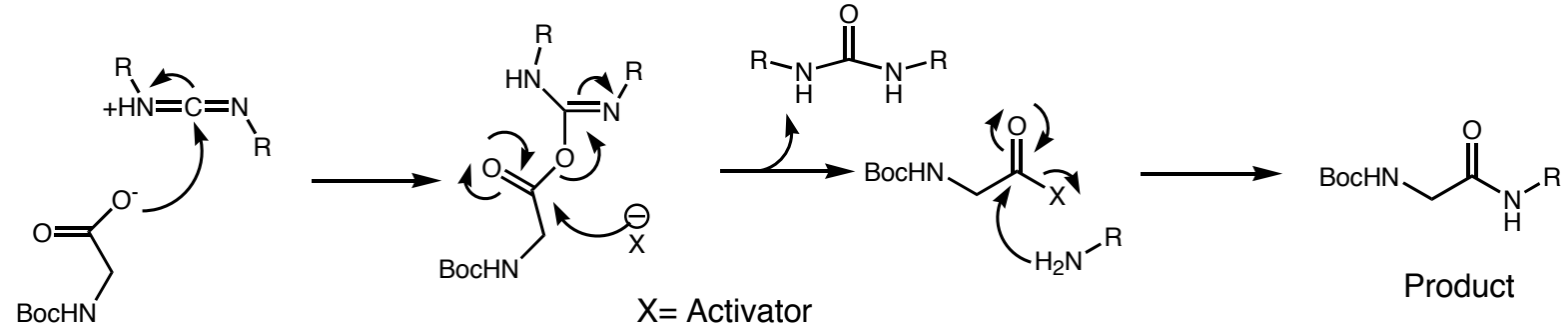




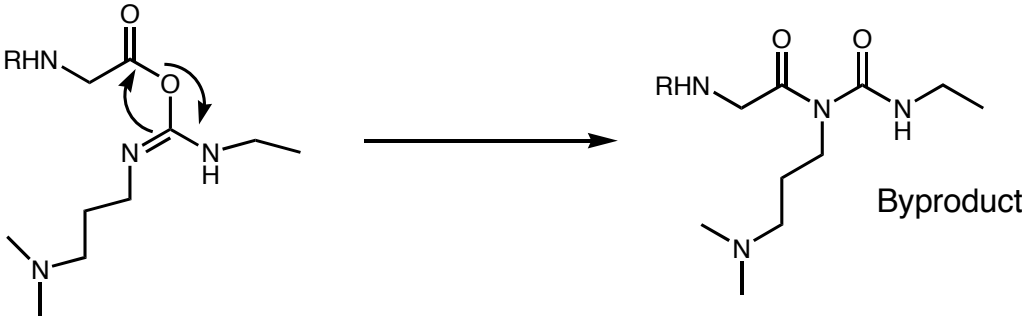
N,N-dicyclopentylcarbodiimide



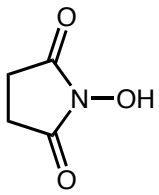
Carbodiimides: Basic Structure and Mechanism



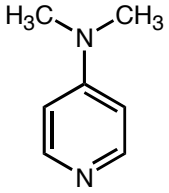
N-acyl Urea Formation



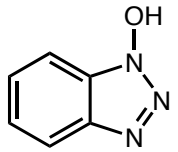
Common Activators: Accelerate Reaction and Suppress Byproduct Formation



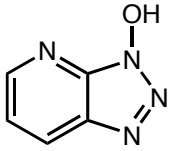
HOSu



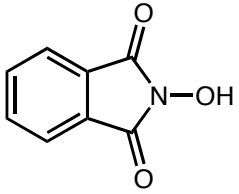
DMAP



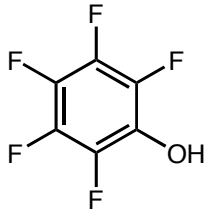
HOBt



HOAt



N, hydroxyphthalimide



PfpOH

Lou Carpino: Peptide Giant
UMass, Amherst

- Developed benzotriazole based aminium reagent, HATU, and elucidated the active form of the coupling agent

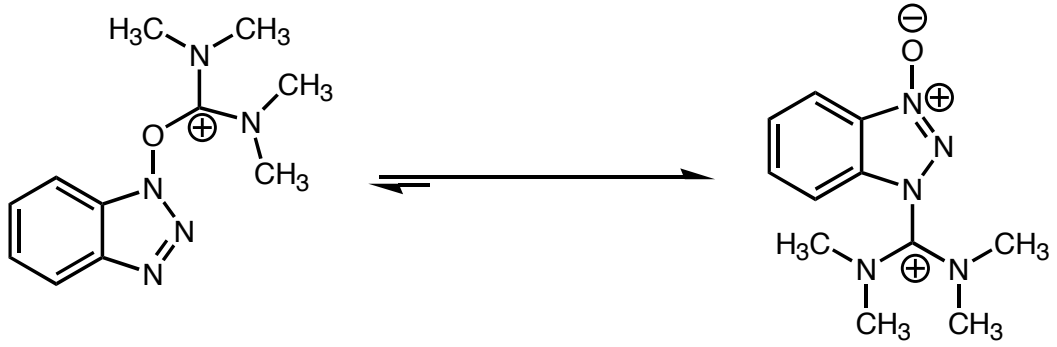
- Introduced HOAt as an efficient additive for coupling reactions

-Introduced the widely used Fmoc protecting group

-Pioneered the use of amino acid fluorides as coupling agents

Uronium reagents: Basic Structure and Reactive Species

HBTU



Uronium **Guanidinium**

- Originally the uronium isomer was thought to be the active species

-Upon solving of the x-ray crystal structure, it was found that the guanidinium species was predominate

-However, the uronium could be prepared and was found to be more reactive than the guanidinium salt

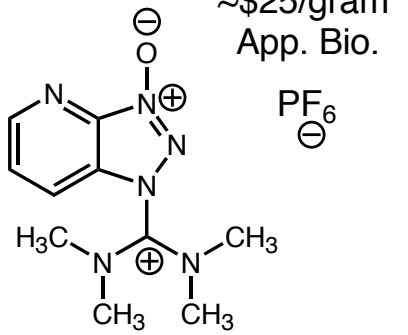
- Original attempts to classify the reactive species were misguided based on known thermodynamic stabilities

-The two forms are readily distinguished by a shift in the IR absorption from ~1710 cm^{-1} (Uronium) to ~1670 cm^{-1} (guanidinium)

J. Org. Chem. **2001**, *66*, 5245-5247
Angew. Chem. Int. Ed. **2002**, *41*, 442

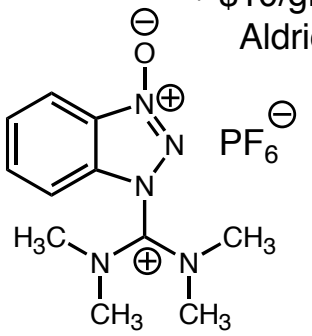
Uronium reagents: Overview/Cost Analysis

~$25/gram
App. Bio.



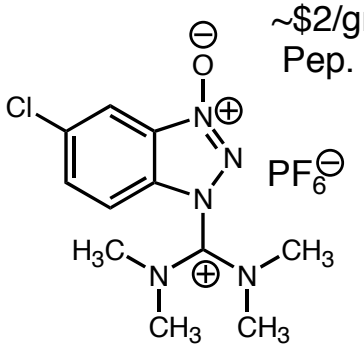
HATU

~$10/gram
Aldrich



HBTU

~$2/gram
Pep. Intl.



HCTU

-HATU is the most reactive uronium reagent, however it can be cost prohibitive on large scales and is often used only as a last resort

-HBTU is the more cost effective alternative and is acceptable for most coupling applications, however lower yielding couplings can become problematic on industrial scales and with long peptides

- HCTU has been developed as an effective alternative to HATU on industrial scales, the higher reactivity of this species is attributed to the more reactive Cl-HOBt intermediate

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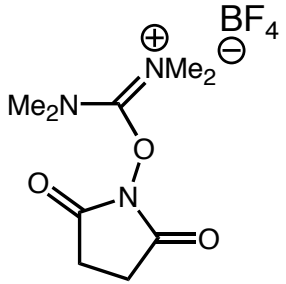
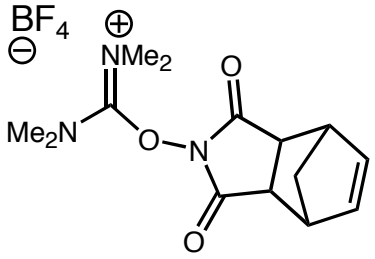


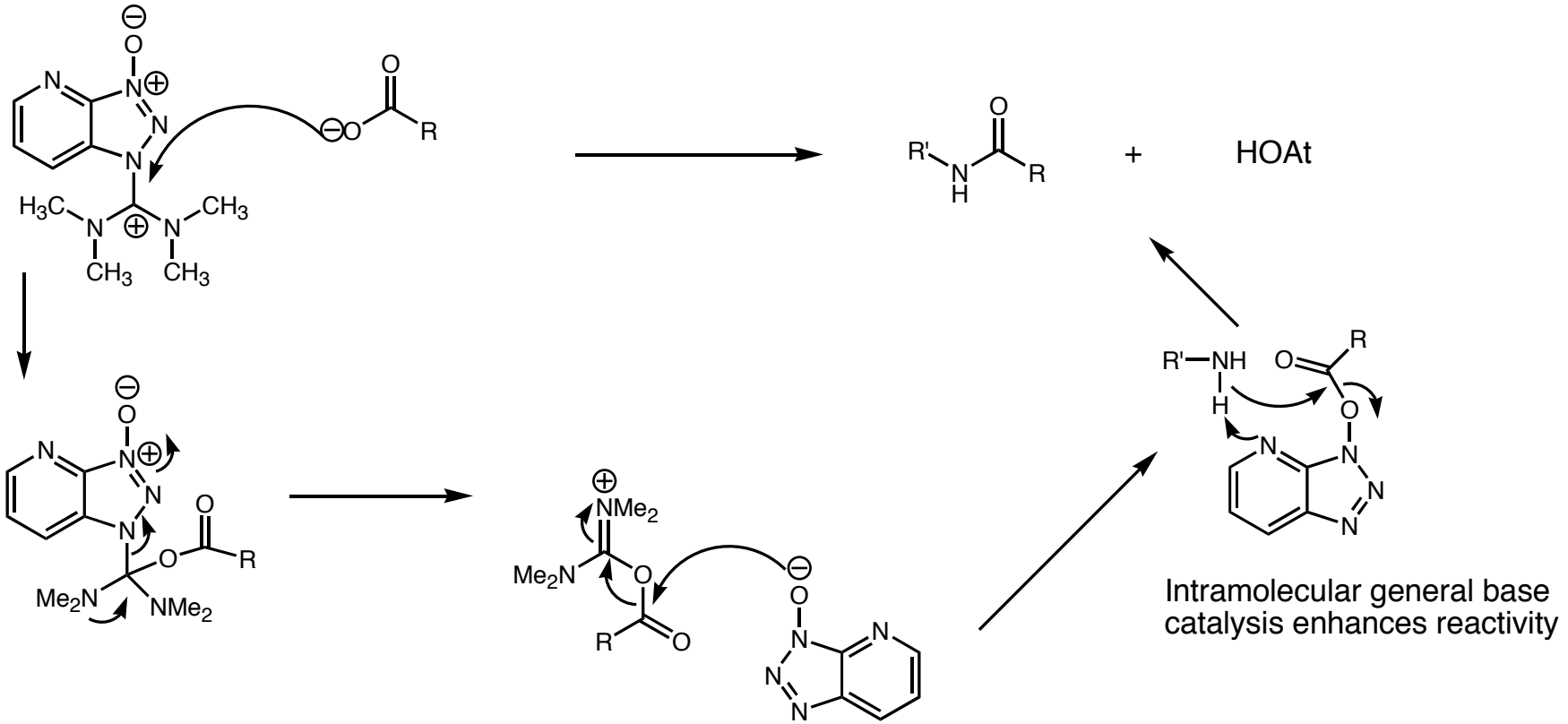
TSTU

- TSTU and TNTU are useful alternatives under aqueous reaction conditions

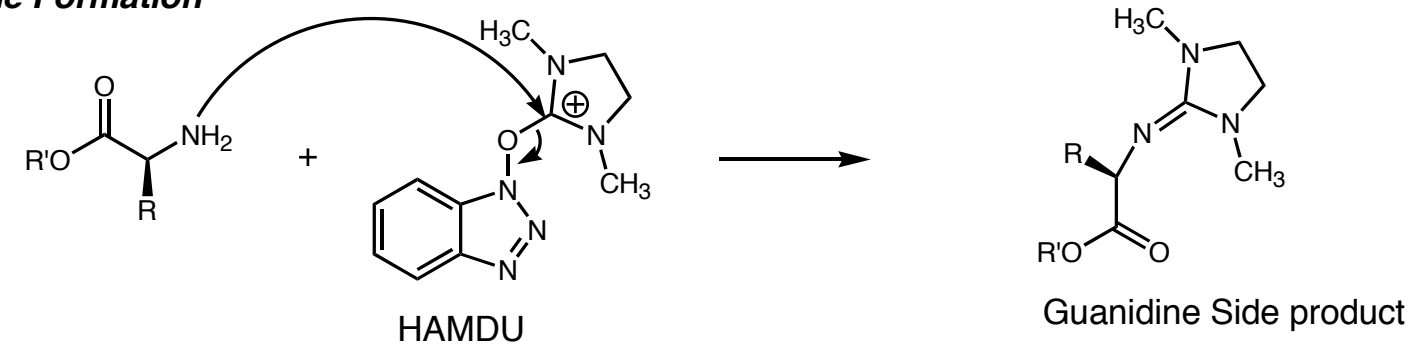


TNTU

Uronium reagents: Mechanism and Side Reactions

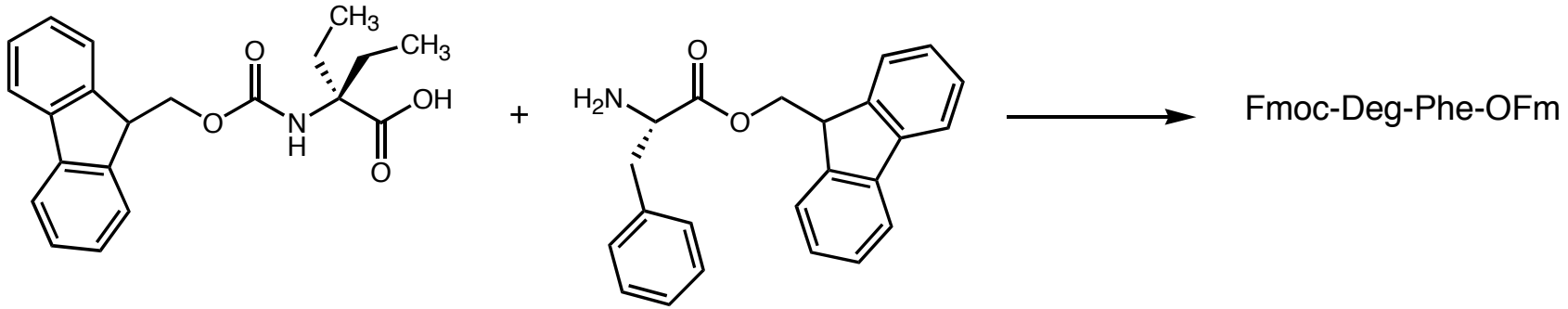


Guanidine Formation



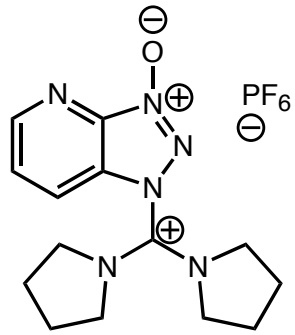
This side reaction highlights the importance of stoichiometry and pre-activation of the acid component. This can generally be avoided with the proper precautions.

Uronium reagents: Efficiency Comparison

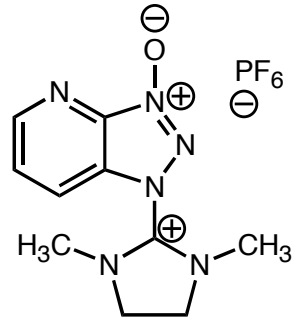


Coupling Agent	HPLC Yield
HATU	94
HBTU	85
HAPyU	92
HAMDU	57
HDTU	64

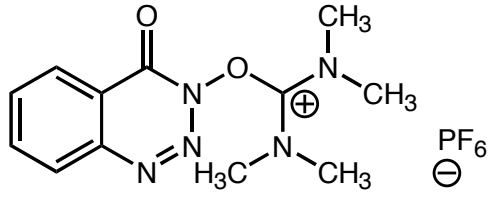
J. Org. Chem. **1998**, *63*, 9678-9683



HAPyU



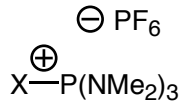
HAMDU



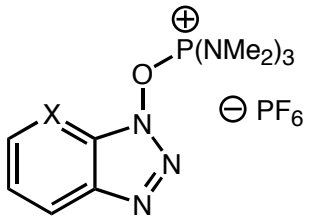
HDTU

Phosphonium Reagents: The Basics

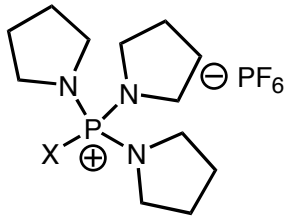
Common Reagents



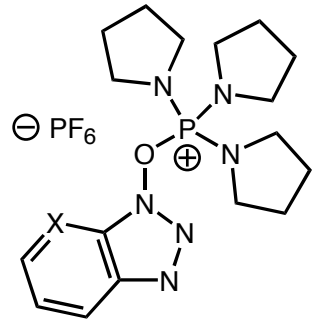
X=Cl, CloP
X=Br, BroP



X=CH, BOP
X=N, AOP



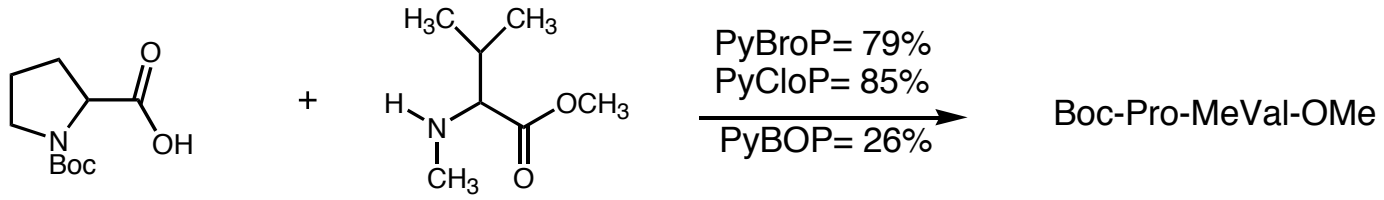
X=Cl, PyCloP
X=Br, PyBroP



X=CH, PyBOP
X= N, PyAOP

- Reagents bearing the dimethyl amine moiety produce HMPA as a toxic byproduct, and thus their pyrrolidine based analogues are preferred

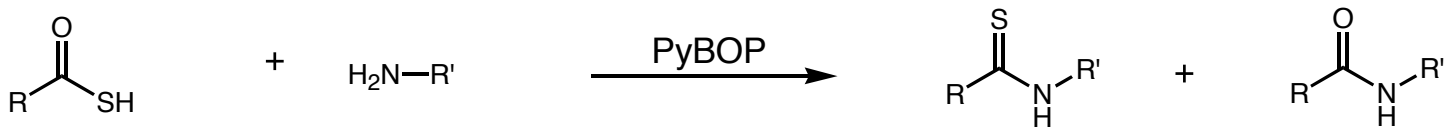
- Halogenophosphonium reagents have been shown to be more efficient coupling reagents in the coupling of N-methylated amino acids



"Difficult" because of steric hindrance

J. Org. Chem. **1994**, *59*, 2437-2446

Phosphonium Reagents: Thioamide Formation



2.5 : 1

(68% overall yield
of both products)

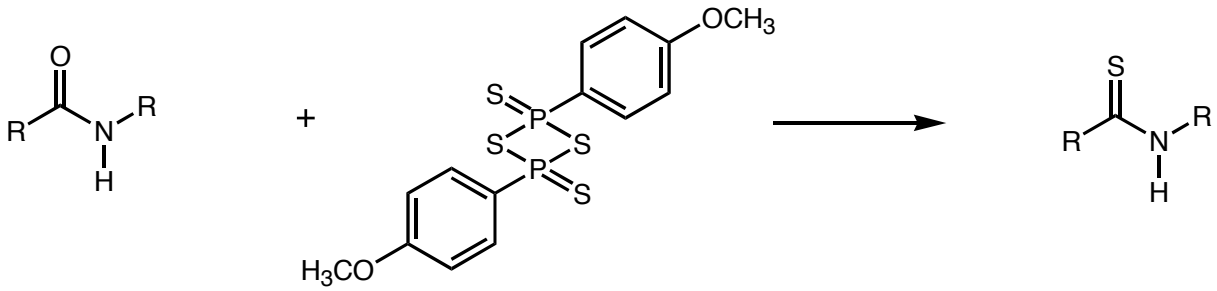
- Thioamides are useful probes in peptide structure and function, particularly for the elucidation of the contribution of backbone hydrogen bonding

- These peptide analogues are not readily accessed through thioacylation in an analogous fashion to standard peptides

- This example uses the oxophillicity of the phosphonium coupling reagent to direct thioamide formation preferentially over amide formation

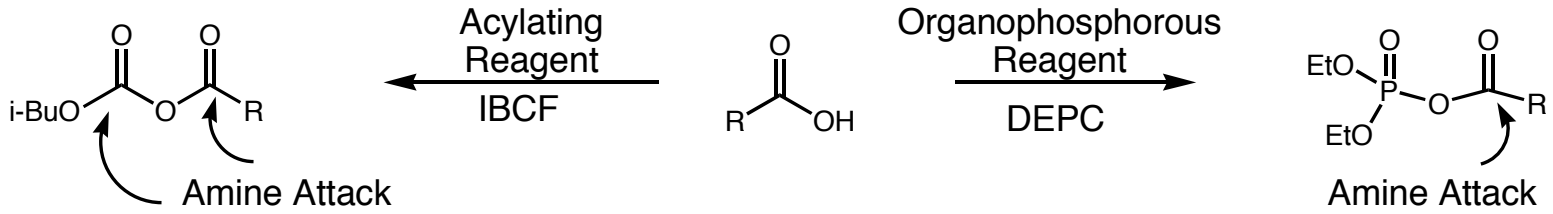
-Interestingly, this selectivity is reversed (1:24) with the use of PyBrOP

Note: Thioamides are also readily prepared using Lawesson's reagent



J. Org. Chem. **1994,** *59*, 1257-1263

Organophosphorous Reagents



- Originally developed as an alternative to the mixed anhydride method

-The use of organophosphorous reagents gives enhanced regioselectivity toward the carbonyl of the phosphoric-carboxylic mixed anhydride

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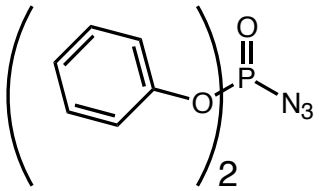
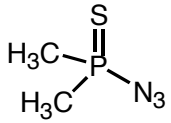
Common Organophosphorous Reagents



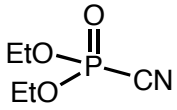
DPPA

Oil, difficult to handle



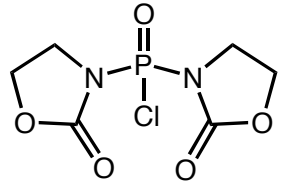
MPTA

Crystalline, stable



DECP

Useful for nucleophillic amines

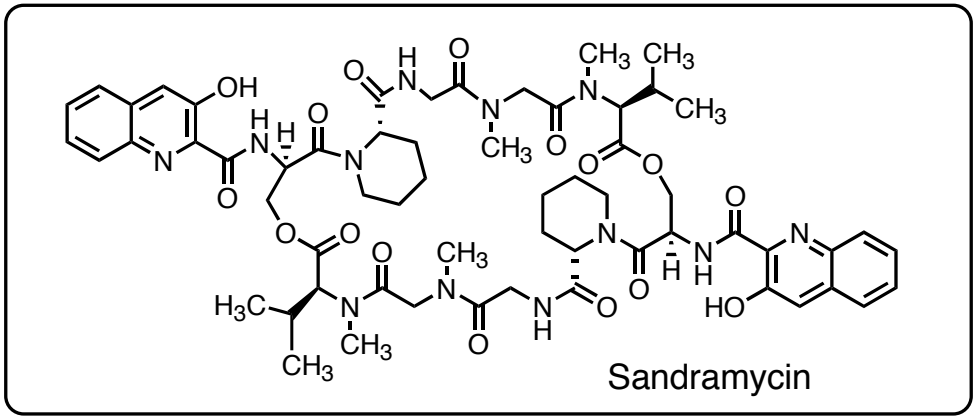


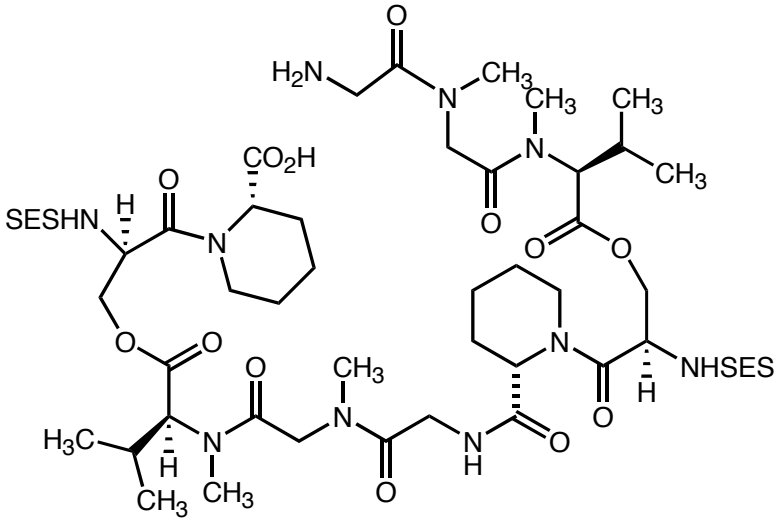
BOP-Cl

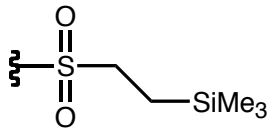
Especially good for N-alkyl amino acids

Organophosphorous Reagents: Practical Considerations

Boger's Total Synthesis of Sandramycin

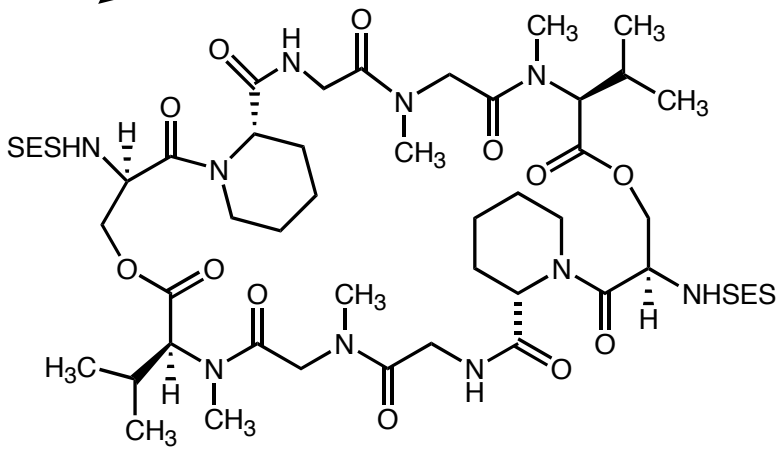




SES= 

DPPA, $NaHCO_3$

90%

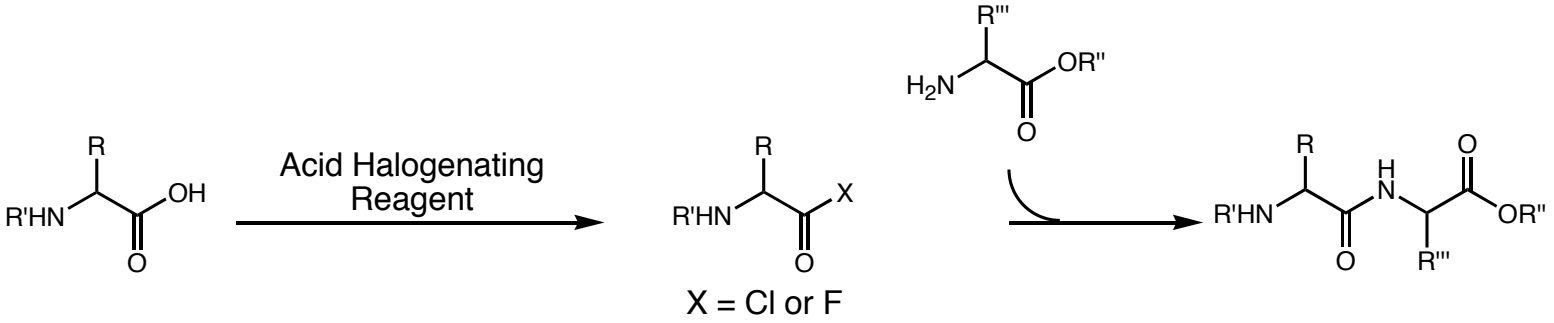


- Macrocyclization was accomplished under mild conditions using $NaHCO_3$ as a base

-Use of stronger bases led to decomposition

J. Am. Chem. Soc. **1996,** *118,* 1629-1644
J. Org. Chem. **1987,** *52,* 764-769

Acid Halogenation

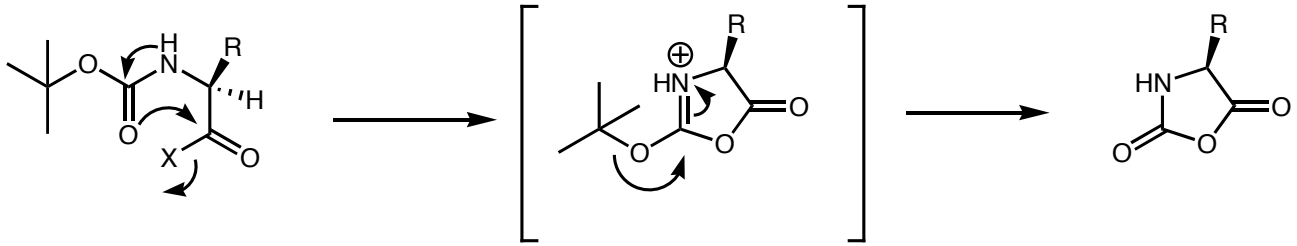


- Acid halides are typically used when steric congestion prohibits the use of standard coupling reagents

- Currently there are a number of commercially available Fmoc amino acid fluorides, however Boc and Cbz groups present problems in the coupling of acid halides

- For difficult couplings the more reactive and less expensive acid chloride would be preferred, however until recently an appropriate protecting group was not available

Carboxyanhydride Formation: An Unwanted Side reaction



X=Activator

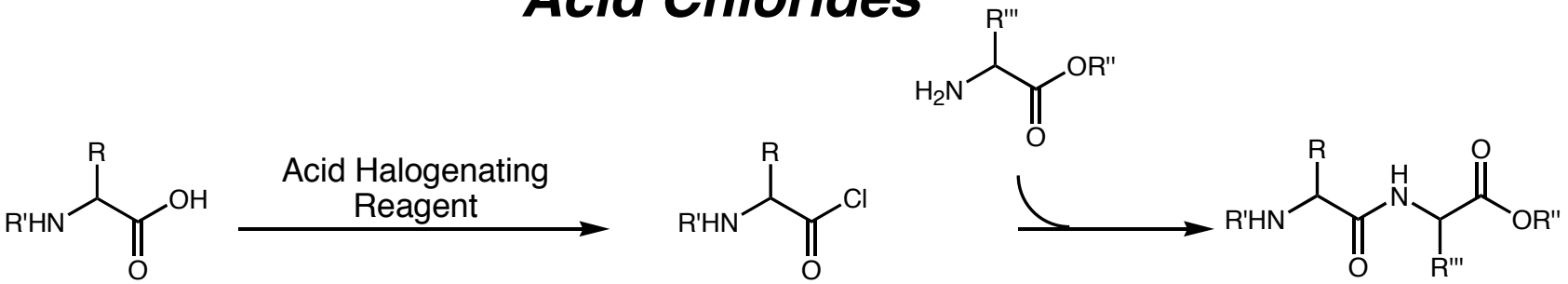
Oxazolonium Ion

N-carboxyanhydride

This side product can be significantly reduced with careful selection of protecting groups for the amine functionality. Boc protected amines form the carboxyanhydride byproduct much more readily than the corresponding Fmoc or Cbz amino acids.

Acc. Chem. Res. **1996,** *29*, 268-274
J. Am. Chem. Soc. **1996,** *118*, 9796-9797

Acid Chlorides



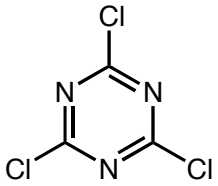
- Acid Chlorides were first introduced for peptide coupling in 1903 by Emil Fischer

-However, when the amine functionality is protected as a carbamate, the oxazolinone is readily accessed, leading to racemization and undesirable side products

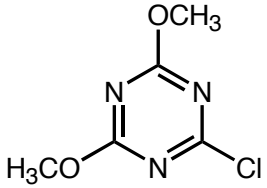
-These problems can be avoided by the use of a sulfonyl protecting group, but deprotection conditions may be too harsh for many peptides

-This problem was solved by Vedejs and coworkers

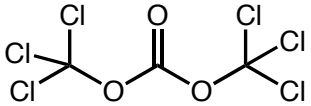
Common reagents to Make Acid Chlorides



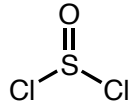
Cyanuric Chloride



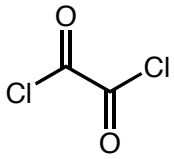
CDMT



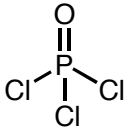
BTC



Thionyl
Chloride

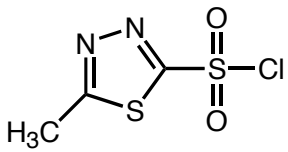


Oxalyl Chloride

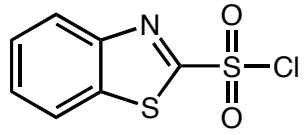


Phosphorous oxychloride ($POCl_3$)

Acid Chlorides: New Sulfonyl Protecting Groups



ThsCl
"thisyl chloride"

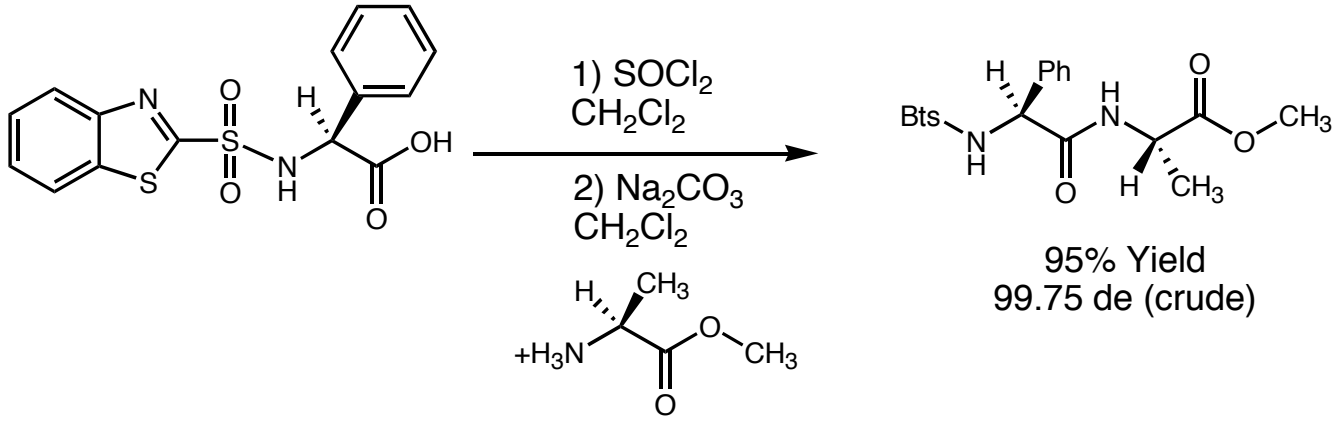


BtsCl
"betsyl Chloride"

- Both sulfonyl chlorides are readily accessed from the commercially available mercapto derivatives

-The corresponding sulfonamides are synthesized in high yield for a number of amino acid derivatives, inculding zwitterionic amino acids

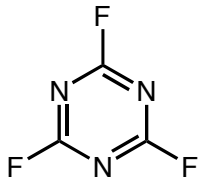
- Both groups can be selectively removed in the presence of other sulfonamides under reducing conditions including; Zn/HOAc, Al-Hg/ether, and H_3PO_2



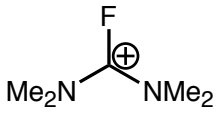
J. Am. Chem. Soc. **1996,** *118*, 9796-9797

Acid Fluorides

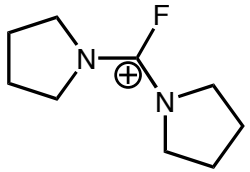
Common Acid Fluorinating Reagents



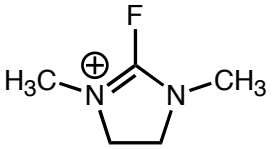
Cyanuric Fluoride



TFFH

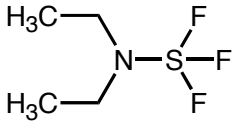


BTFFH



DFIH

Useful for *in situ* generation of acid fluorides in coupling reactions



DAST

DAST can be used in the absence of base to promote acid fluoride formation. Particularly useful in preparing Fmoc amino acid fluorides.

Advantages/Disadvantages of the Approach

- Promotes the formation of amide bonds at sterically hindered sites

- Most carbamate protected amino acids are stable compounds that do not form the N-carboxyanhydride byproduct

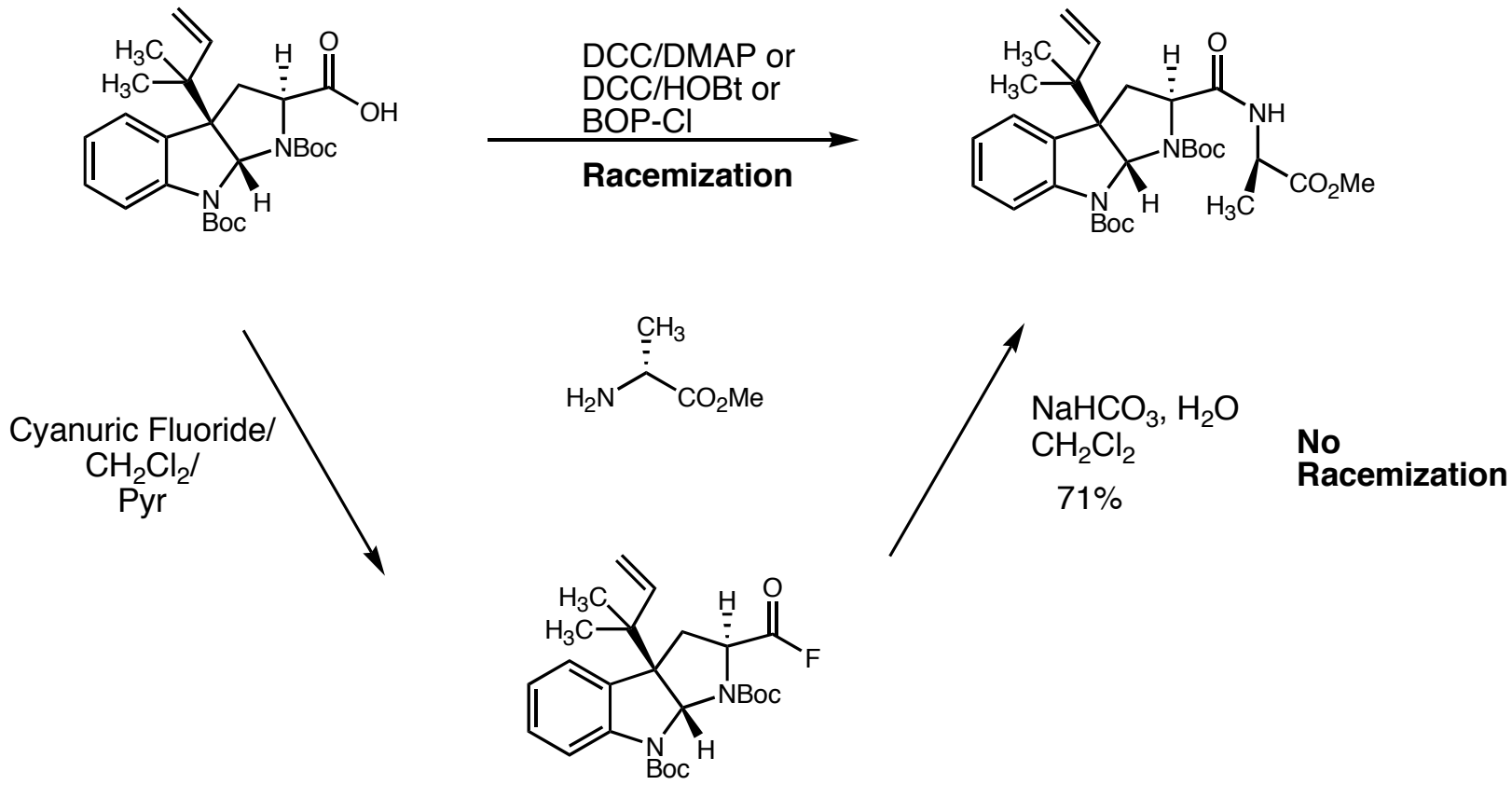
-More water stable than the corresponding acid chlorides

-Arg and His are not stable and need to be generated *in situ*

- The amino acid fluorides show similar reactivity to activated esters and may not be appropriate for difficult couplings at unreactive sites

J. Am. Chem. Soc. **1995**, *117*, 5401-5402
Lett. Pept. Sci. **1996**, *2*, 285

Acid Fluorides Applied

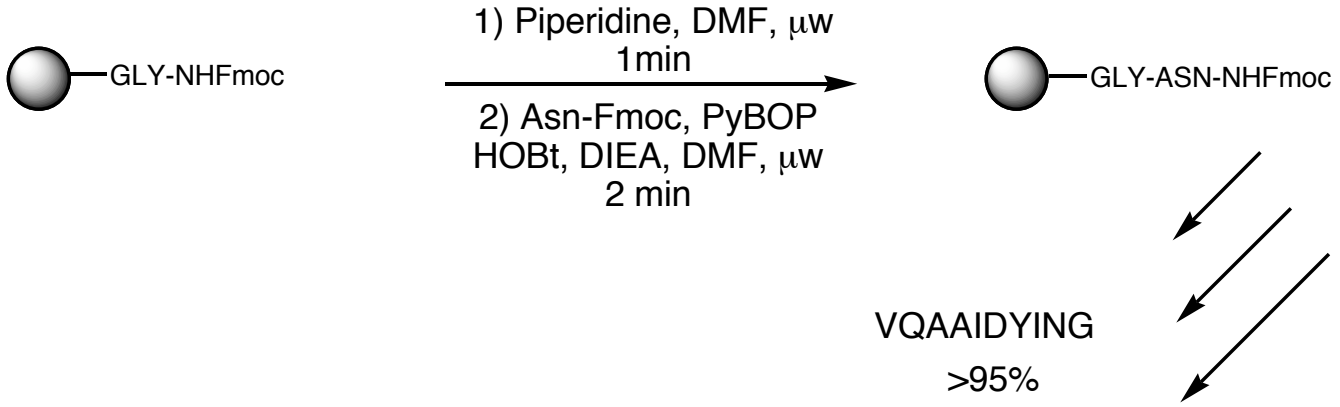


- Danishefsky and co-workers applied an acid fluoride mediated coupling in route to 5-N-acetylardeemin when standard coupling reagents failed to produce a diastereomerically pure compund

J. Am. Chem. Soc. **1999**, *121*, 11953-11963

Microwave Assisted Synthesis

Solid Phase Synthesis of the Acyl Carrier Peptide



- Researchers at CEM Corporation were able to rapidly and efficiently assemble a decameric peptide in >95% overall yield with microwave assisted synthesis

- The reactions were enhanced through microwave radiation allowing for higher resin substitution, less reagent excess and higher coupling yields due to decreased intramolecular aggregation

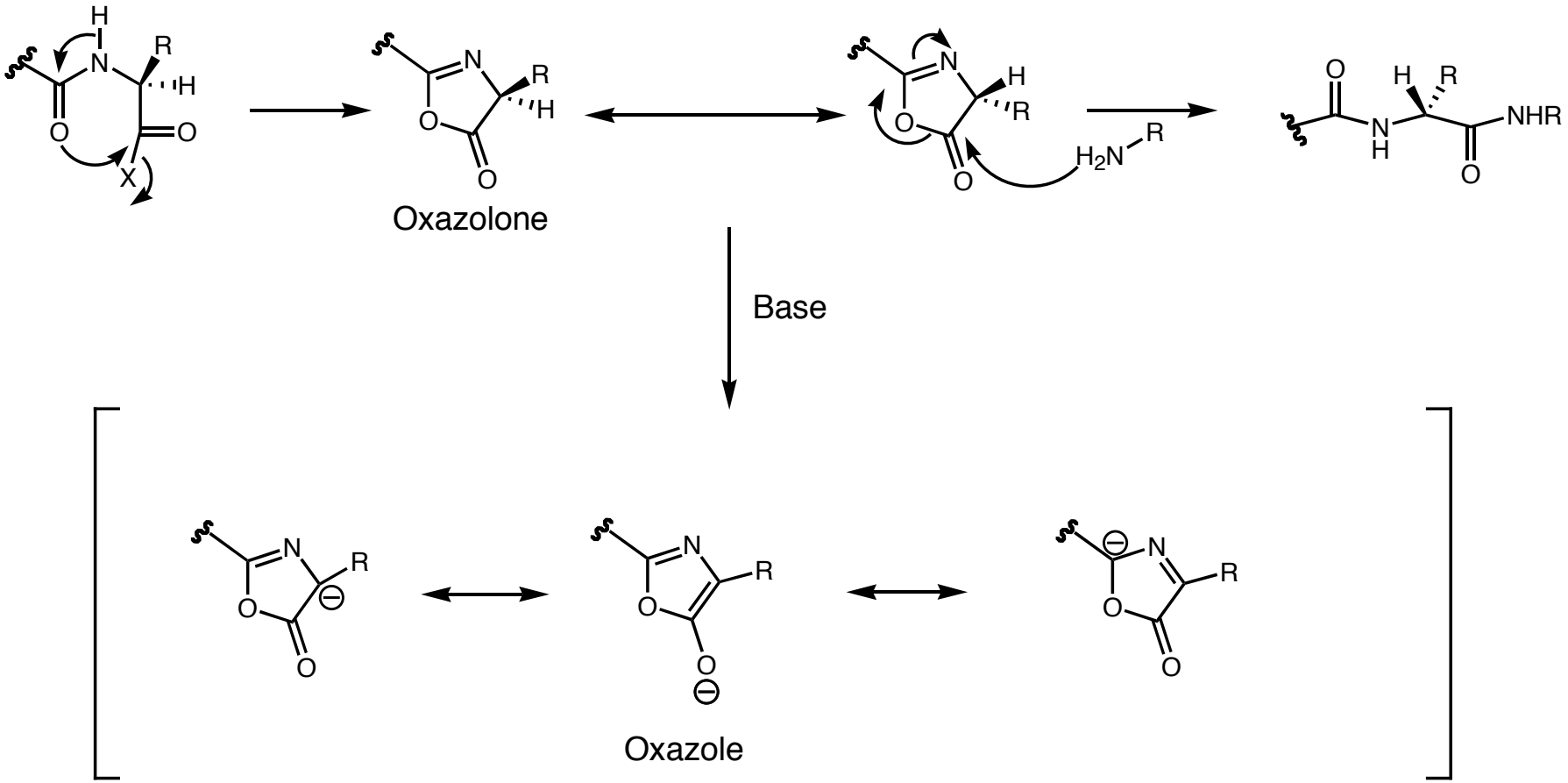
Biopolymers, **2003,** *71,* 361

Reagent	PyBOP	HATU
Reaction Time(min)	20	1.5
Temperature(^{o}C)	110	110
Solvent	DMF	DMF

Synthesis, **2002,** *11,* 1592-1596

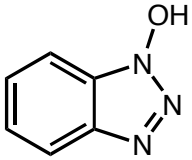
Racemization Pathways

Oxazolone Formation



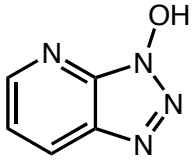
Epimerization can be controlled with the appropriate rate enhancing, racemization suppressants

Racemization Suppressants



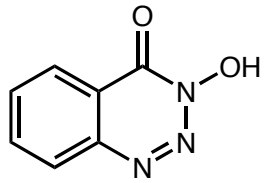
HOBt

Most commonly used racemization suppressant. Often used in combination with carbodiimide chemistry



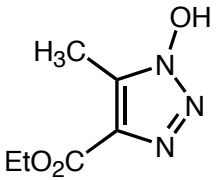
HOAt

HOAt is more effective than HOBt due to intramolecular general base catalysis. Caution should be used on large scales, as HOAt is slightly explosive



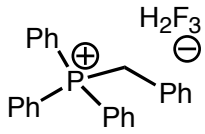
HODhbt

HODhbt has yet to find widespread use due to a ring opening side reaction



HOCt

When used with DIC, HOCt suppressed all racemization except with histidine.



PTF

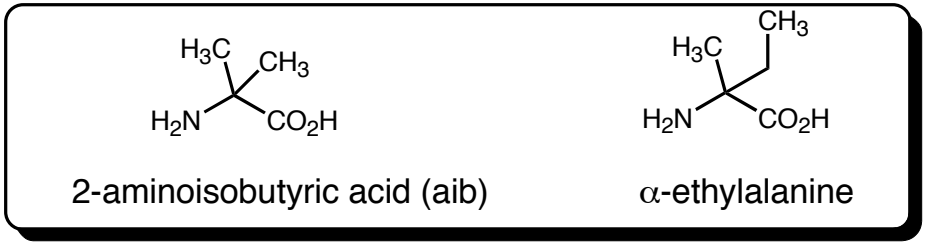
When PTF is used in combination with HBTU, the coupling efficiency is equivalent to that of HATU. Unsuitable for phosphorous reagents because of strong P-F bond

Org. Lett. **2003,** *5*, 975-977

- Racemzation suppression agents also work to enhance the reactivity of intermediates in the coupling reaction

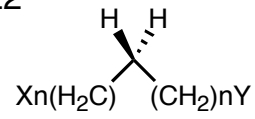
-Also, Cu(II) salts have been found to act as racemization suppressants in the presence of standard coupling agents ($CuCl_2$, $Cu(OBt)_2$, $Cu(OAt)_2$), *J. Pept. Sci*. **2001,** *7*, 115-120

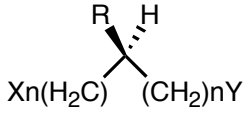
Difficult Couplings: α,α dialkyl

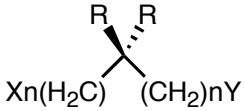


2-aminoisobutyric acid (aib) α-ethylalanine

Synthesis, **1995,** 1205-1222







Calculated Bond Angle: 111.9° 108.4° 106.5°

- Disubstituted amino acids are particularly challenging because of the propensity for racemization of the penultimate residue, and diketopiperazine formation

-Steric congestion also makes these residues less reactive to standard peptide coupling techniques

-Both of these are a result of the geminal dialkyl effect

-Dialkyl substituents force the peptide from it's preferred straight chain, anti-periplanar conformation into a staggered conformation, decreasing the bond angle in the chain and facilitating ring formation

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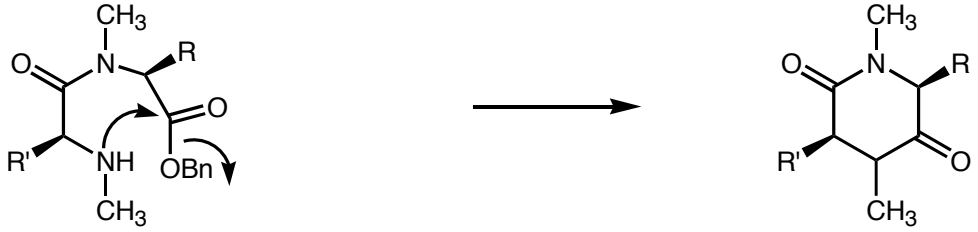
Diketopiperazine Formation

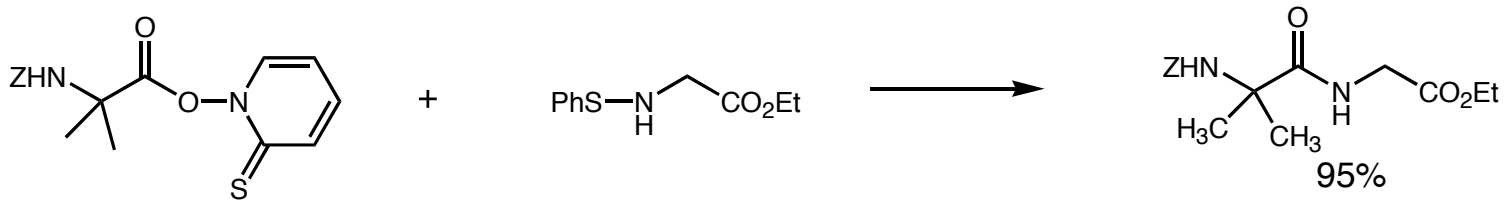


Diketopiperazine

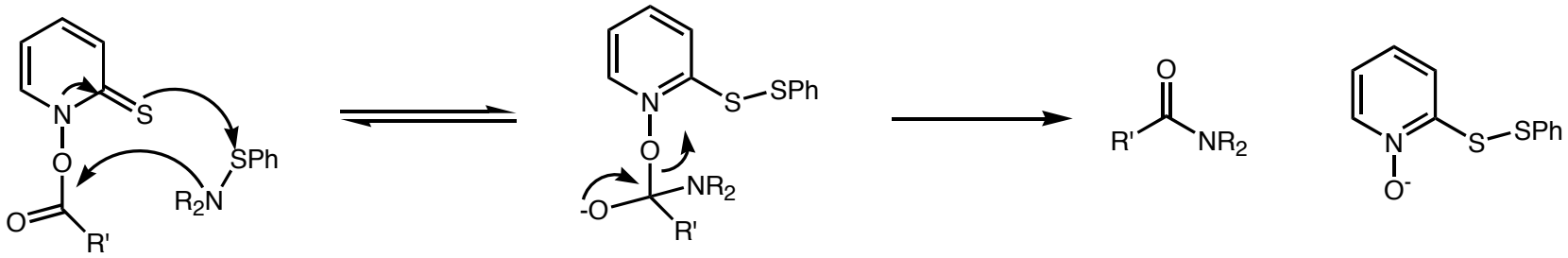
Diketopiperazine formation is especially problematic in the synthesis of peptides containing N-methylated amino acids. This is in part due to N-alkyl peptide exhibting a greater preference for the Z-amide.

α,α dialkyl : Solutions

Barton PTOC Ester



Proposed Mechanism



- PTOC esters can be generated in situ and coupled, but for higher yields isolation is recommended

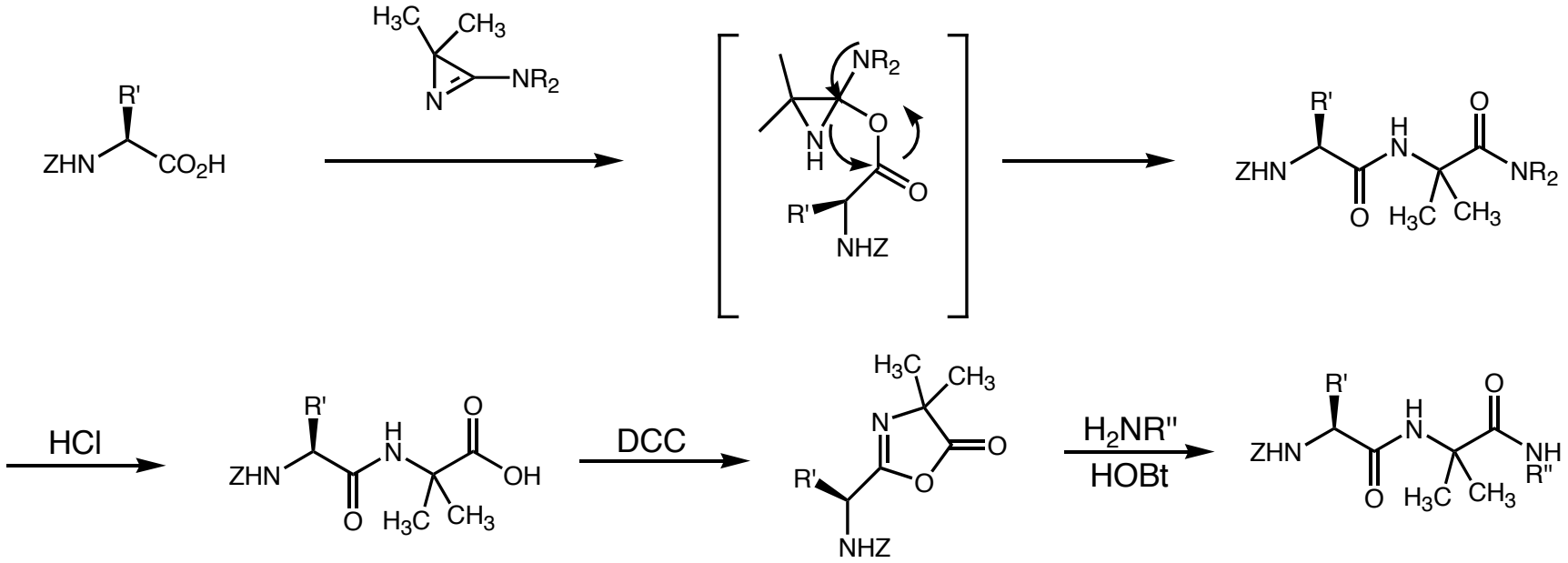
-Coupling proceeds under base free conditions, eliminating racemization

- Free amines can be used in place of sulfeneamides but yields decrease

Tetrahedron, **1996,** *52,* 9367-9386

α,α *dialkyl : Solutions*

Azirine Method for Solution Phase Coupling



-Azirine Method was used in the formation of hindered bonds in the natural product peptide, Alamethicin

-This gave high yields and negligible racemization for the following "difficult" peptides

Z-Val-Aib

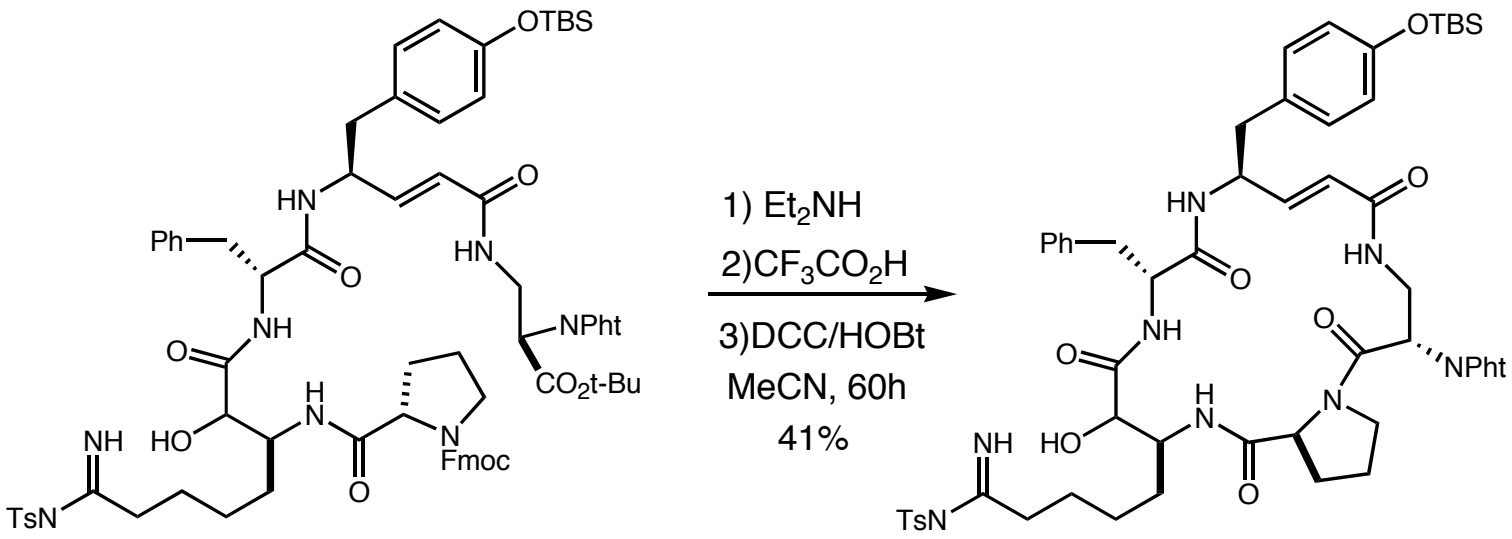
95%

Z-Val-Aib-Aib-NMePh

99%

Helv. Chim. Acta. **1990,** *73*, 13

Carbodiimides: Macrocyclization



Cyclotheonamide A

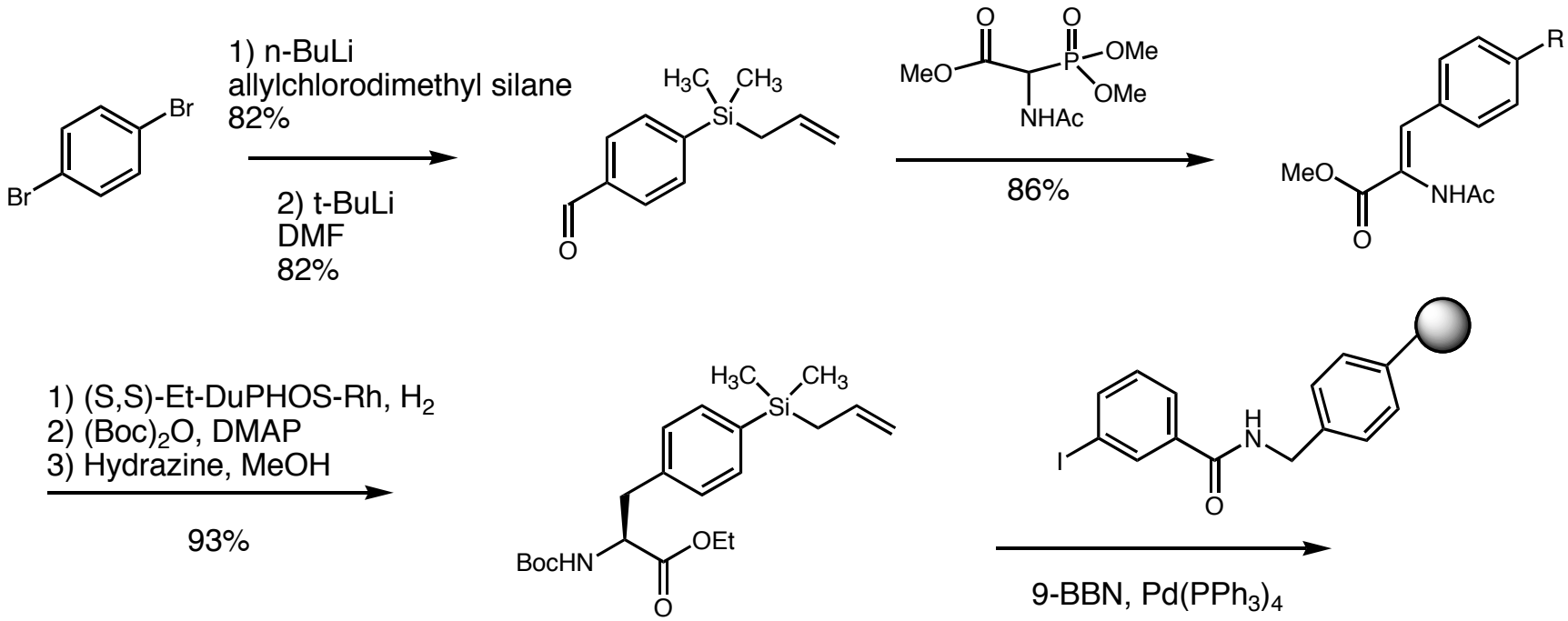
(A serine protease inhibitor, isolated from a marine sponge)

Reagent	Yield
BOP-Cl, DMAP	25
BOP, DMAP	38
EDC, HOBt	24
DCC, HOBt	41
DPPA, $NaHCO_3$	25
BBC, DIEA	36

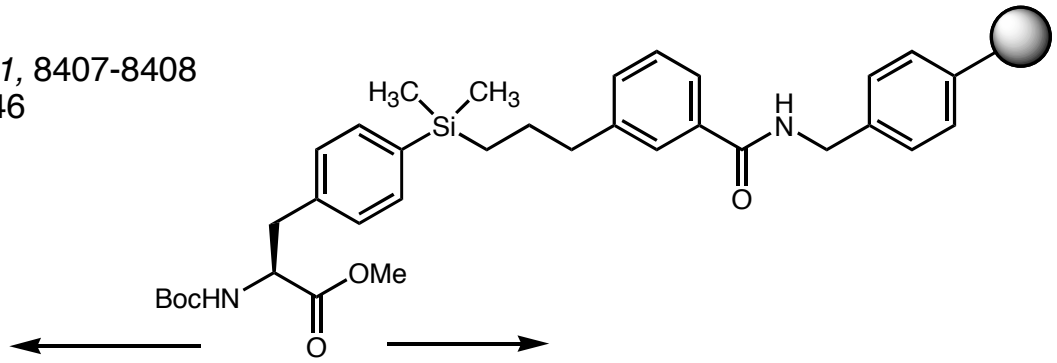
Maryanoff, B.E.; Greco, M.N.; Zhang, H.; Andrade-Gordon, P.; Kauffman, J.A.; Nicolau, K.C., Liu, A.; Brungs, P.H. *J. Am. Chem. Soc*. **1995**, *117*, 1225-1239

Macrocyclizations

Silverman's Traceless Linker



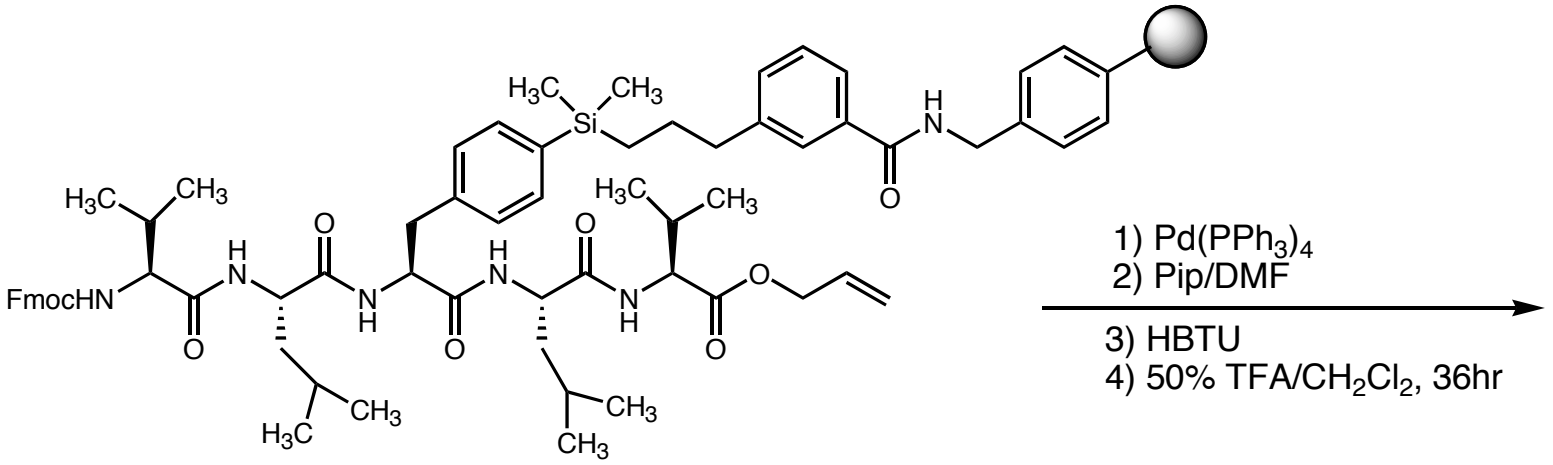
J.Am. Chem. Soc. **1999,** *121,* 8407-8408
Org. Lett. **2000,** *2,* 3743-3746



Orthogonal Proecting groups allow for chain extension in either direction.
Perfect for macrocyclizations

Macrocyclizations

Silverman's On resin Cyclization With a Traceless Linker



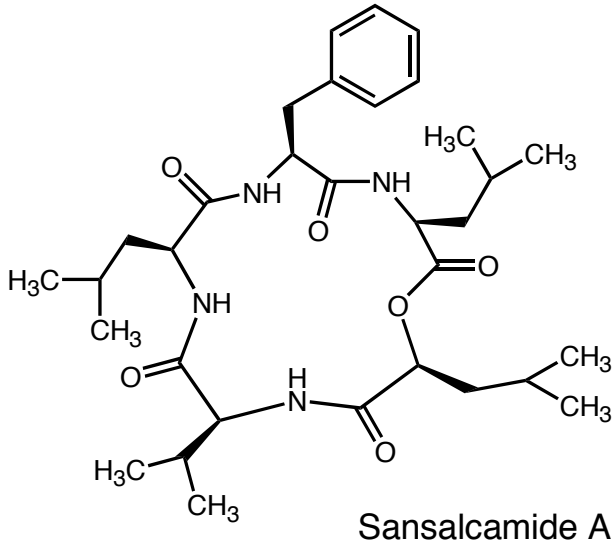
- Silverman applied his side chain attachment methodology to the synthesis of a peptide based natural product, sansalcamide A

- The ten step synthesis was carried out in 67% overall yield and >95% purity

-Alowed for on-resin cyclizations with peptides not bearing polar sidechains

-Solid phase methodology eliminates need for high dilution, and reduces dimerization and oligomerization

-Especially important because many biologically active peptides are exclusively hydrophobic

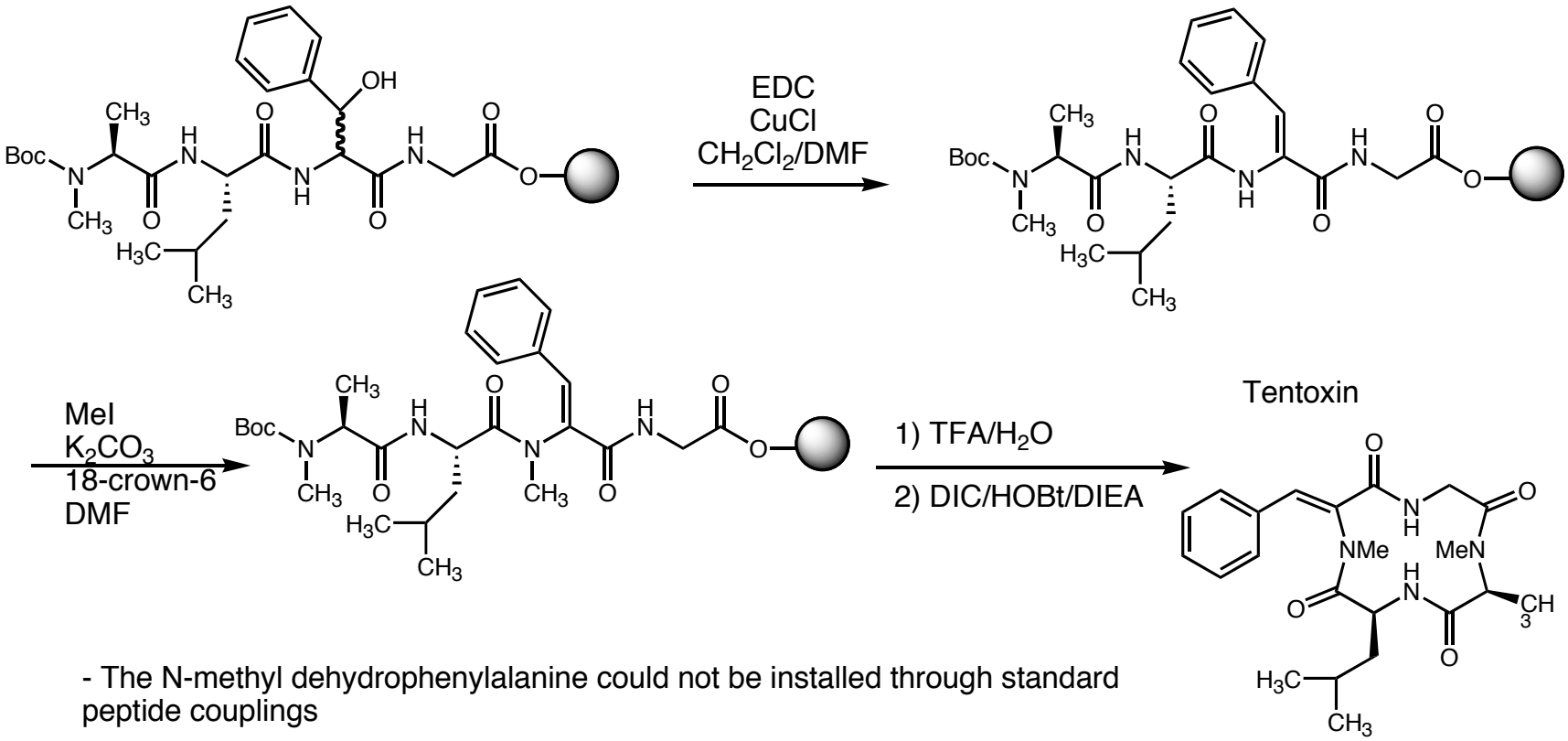


Sansalcamide A

Org. Lett. **2000,** *2,* 3743-3746

Macrocyclizations : Tentoxin

Novel Incorporation of Difficult Residues



- The N-methyl dehydrophenylalanine could not be installed through standard peptide couplings

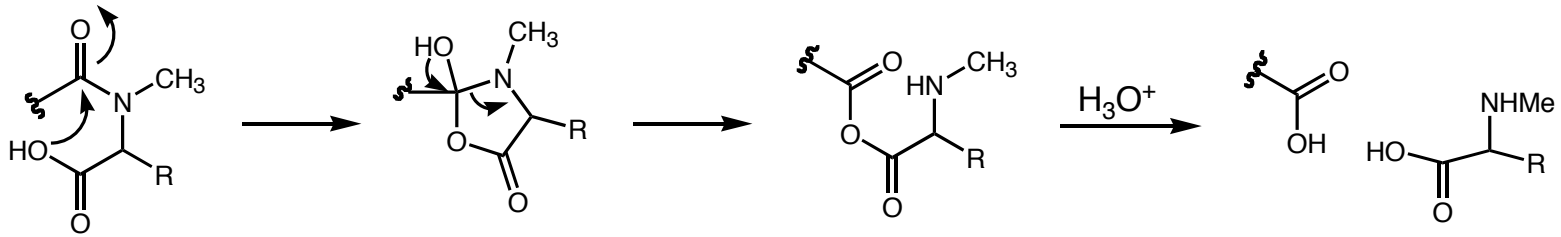
- The dehydro amino acid was installed by an elimination reaction of the unprotected precursor

- N-methylation was also carried out regioselectively on solid support due to the enhanced acidity of the amide proton of the dehydro-residue

Org. Lett. **2003,** *5,* 2115-2118

Difficult Couplings: N-methyl Amino Acids

Acid Catalyzed Cleavage of Imino Acid Sequences



Proceeds through Z-amide conformer. Minor side reaction however and is generally too slow to cause significant problems.

- N-alkyl amino acids are more prone to cleavage and diketopiperazine formation because the Z-amide is more populated than in standard amino acids, making cyclizations favorable

-Furthermore, racemization is more problematic because the alpha proton is the most acidic proton whereas in natural amino acids the amide proton would be deprotonated first

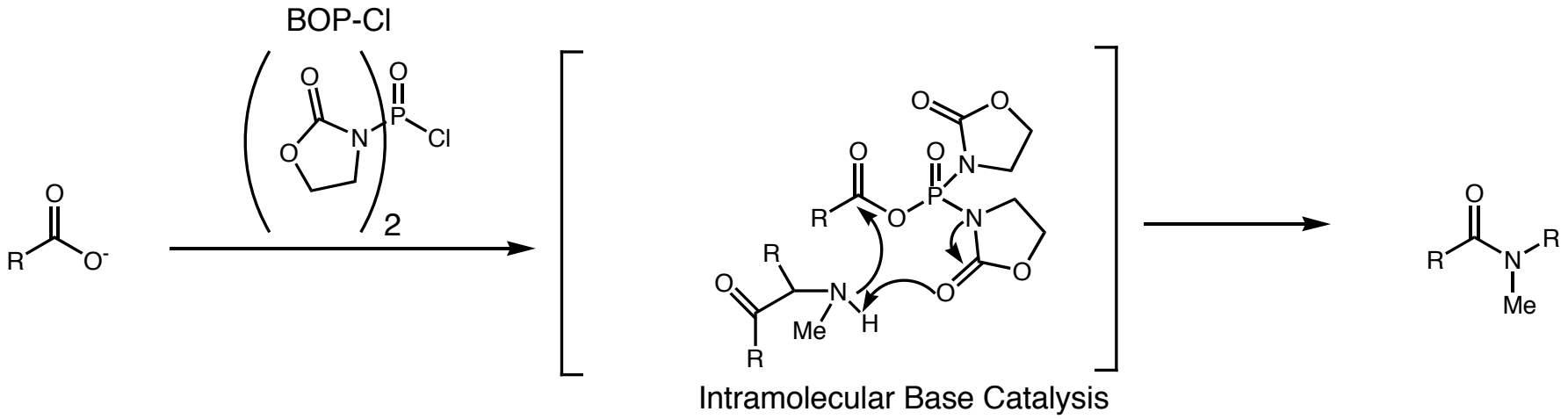
-The steric bulk of N-alkyl AAs reduces the nucleophillicity of the amine, slowing the reaction rate and leading to undesirable byproducts

- It is important to note that in the case of N-methyl AAs, HOBt suppresses the reaction rate and benzotriazole based reagents should be avoided in most cases

Tet. Lett. **1991,** *32,* 1967

N-methyl Amino Acids: Solutions

BOP-Cl as an Effective Coupling Agent of Me-AAs



- Intramolecular base catalysis is proposed to allow for efficient coupling with BOP-Cl where other phosphorous based reagents fail

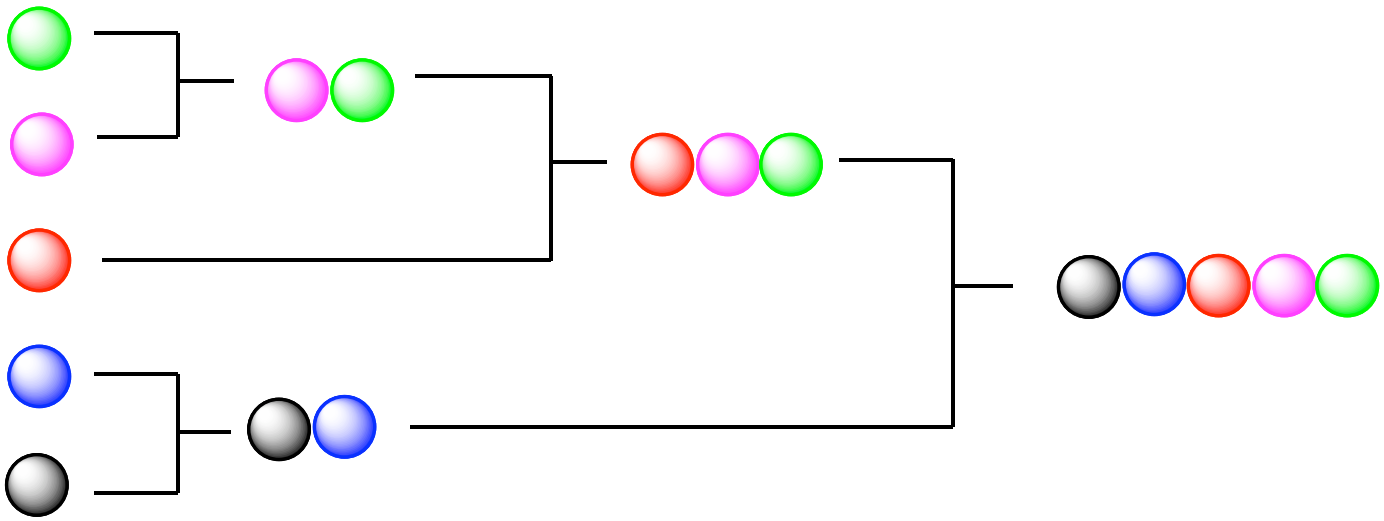
- BOP-Cl allows for one-pot couplings of N-MeAAs because of selective reaction with the carboxylate

-primary amines react with BOP-Cl yielding undesired side products

- It is critical to employ high purity BOP-Cl and is best to prepare it from ethanol, diethylcarbonate and PCl_5, *Bull. Soc. Chim. Belg*. **1986,** *95,* 203

- SPPS does not proceed efficiently with BOP-Cl. HOAt based reagents are the preferred reagents for SPPS of N-methyl AAs and do not suffer the low reactivity of HOBt esters

Segment Condensations



- For large peptides, often times solid phase synthesis is not an operative pathway

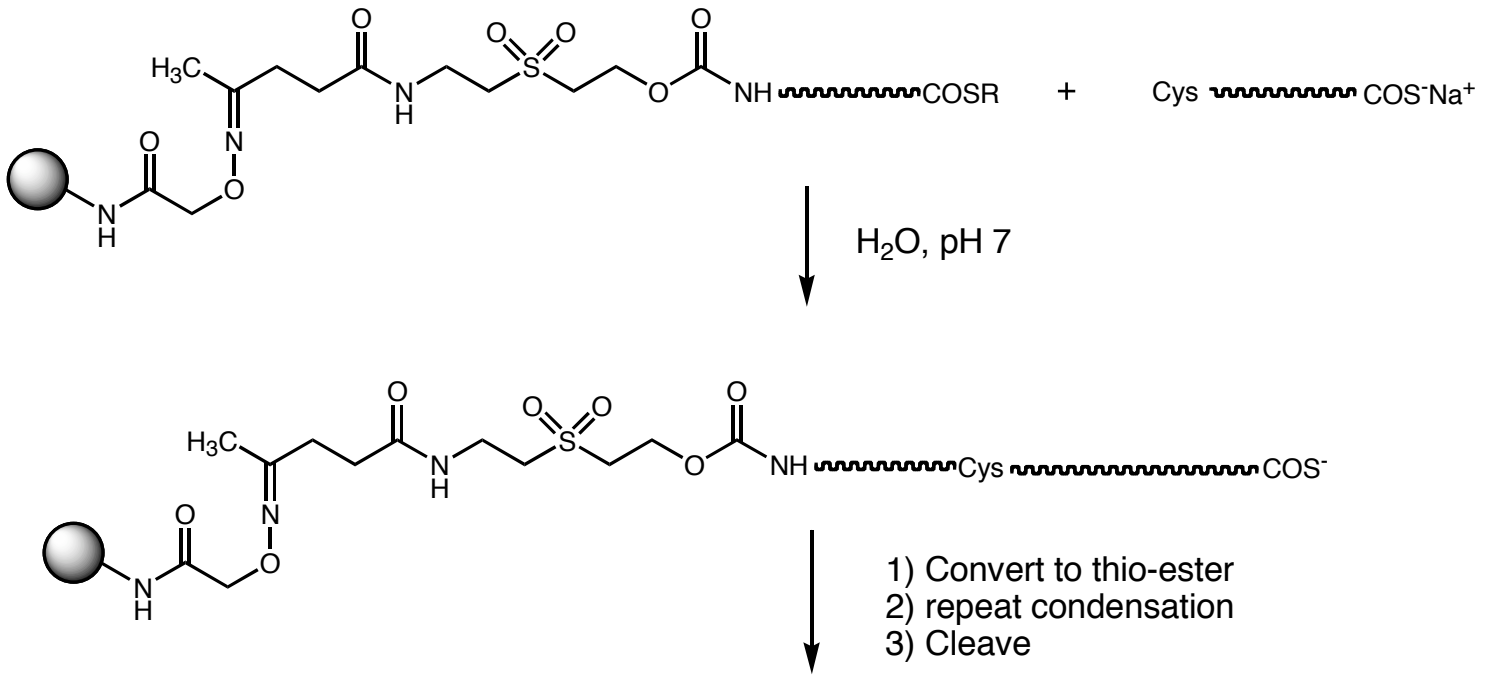
-Peptides/proteins must be made by convergent methods in which smaller pieces are brought together to form the whole oligomer

-There are two ways to accomplish this, solution phase couplings using standard peptide bond forming reagents or native chemical ligation

-Solution phase couplings are often made chemoselective by a protein's propensity for folding, this however requires additional protection strategies if multiple segments are to be coupled

Segment Condensations

Native Chemical Ligation (Kent Ligation)



Peptide

- Solid phase chemcal ligation avoids the need to protect inernal residues within the peptide segments

- Also, tedious purification of intermediates is no longer an issue

- Assembly of the peptide components can proceed in either the C to N or N to C directions, the C to N direction however requires an additonal transient protecting group on the N-terminal cysteine

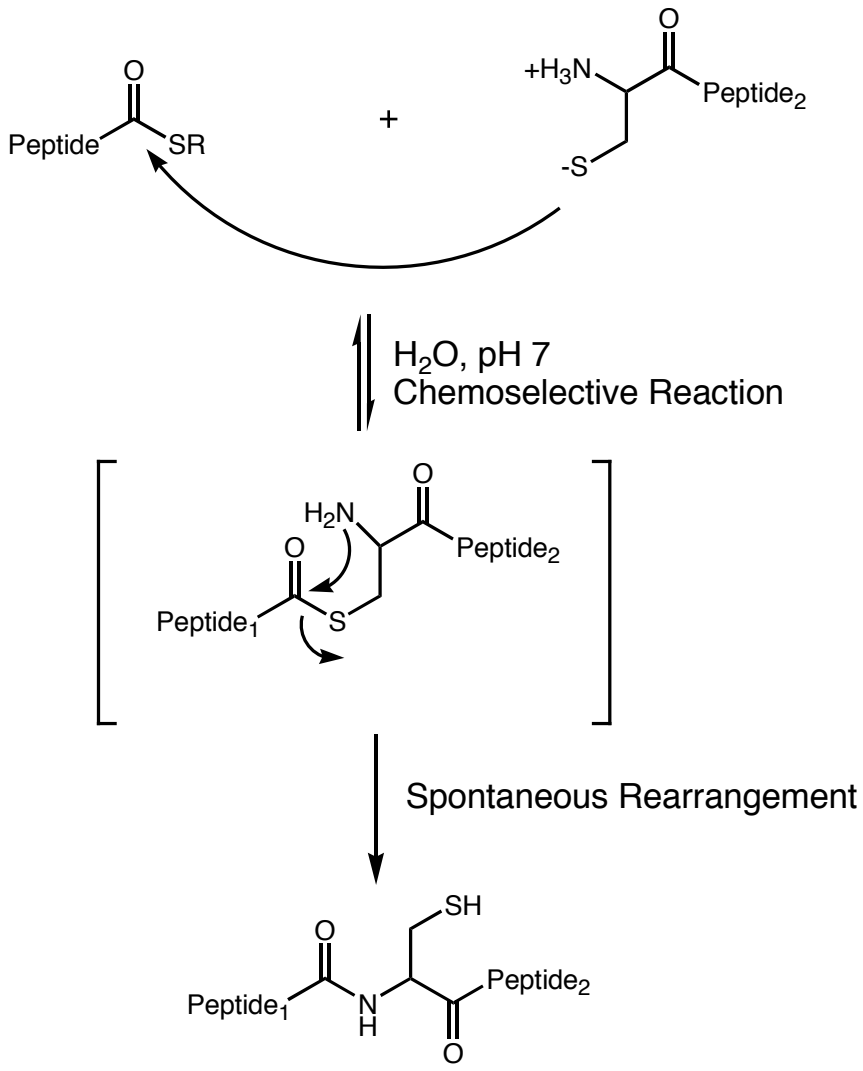
- Racemization is not a problem because peptides are coupled under neutral conditions

- Coupling using a C-terminal thio acid eliminates cyclization products derived from self-condensation

J. Am. Chem. Soc., **1999**, *121*, 8720-8727

Segment Condensations: Mechanism

Native Chemical Ligation (Kent Ligation)

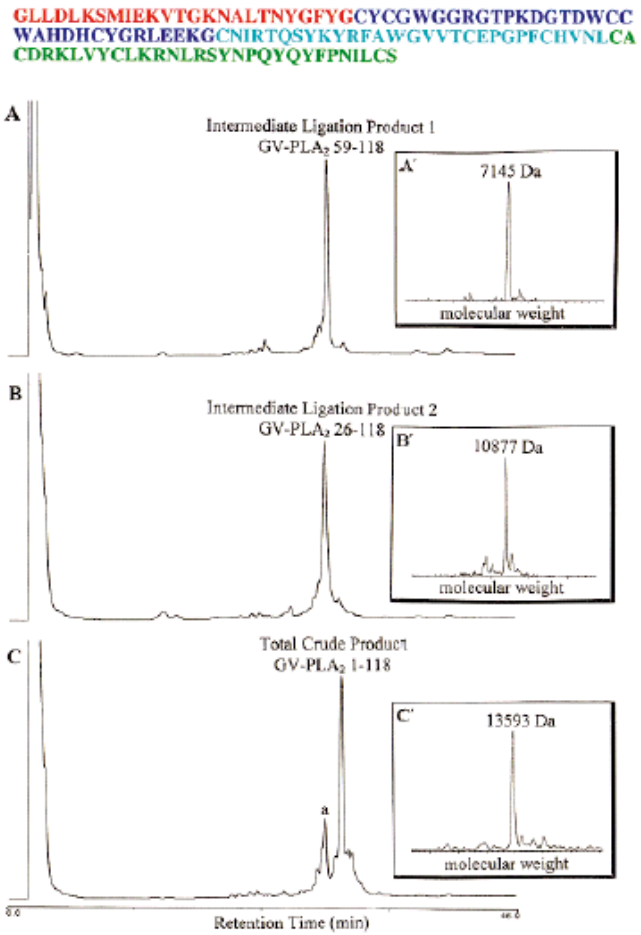


J. Am. Chem. Soc., **1999**, *121*, 8720-8727

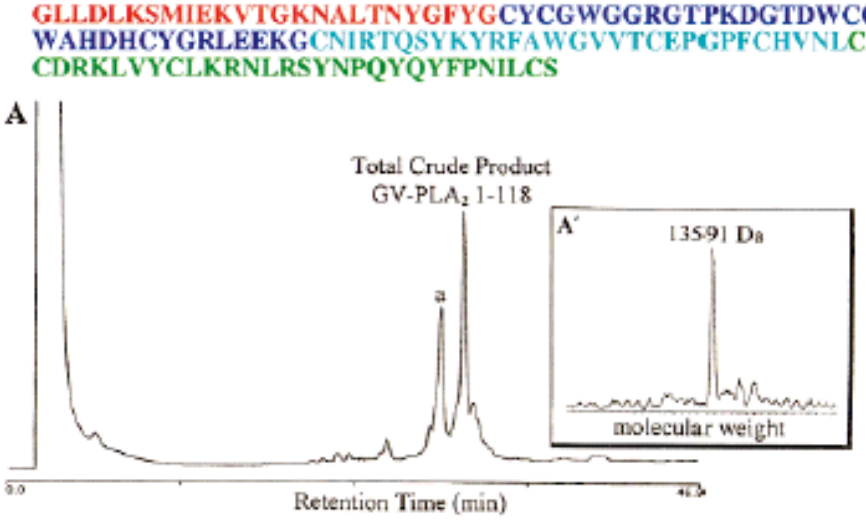
Segment Condensations: Applied

Solid Phase Protein Synthesis

Solid Phase Chemical Ligation (SPCL)



"Accelerated" SPCL



- GV-PLA2, a 118 residue phospholipase was synthesized using SPCl in four segments with three ligation reactions

- The molecule contains six disulfide bonds which could potentially compete with the ligation reaction

- The major peak was collected, found to correspond to the mass of the full length peptide, and shown to possess full biological activity

- An accelerated method was used in which concentrated solutions of each peptide component were used and ligation reactions proceeded for 1 hour, instead of the usual overnight reaction time

J. Am. Chem. Soc., **1999**, *121*, 8720-8727

Segment Condensations: Improved

Chemical Ligation of Non-cysteine Containing Peptides

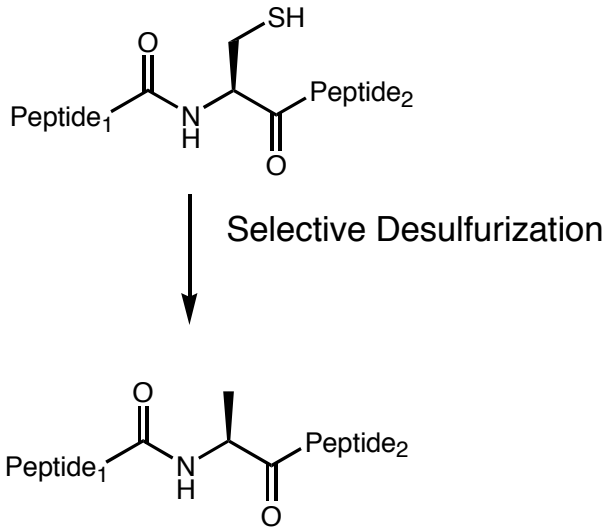


Table 2. Desulfurization Conditions for PGB1

entry	metal reagent	reaction medium	yield (%)	potential problem
1	Pd/Al_2O_3	0.1 M phosphate, pH 5.8, 6 M guanidine	80	hydrogenation of tryptophan
2	Raney nickel	0.1 M phosphate, pH 5.8, 6 M guanidine	82	demethylthiolization of methionine

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- Native Chemical Ligation sequence can be carried out followed by a desulfurization step to afford a ligated product linked at an alanine residue

- Desulfurization readily occurred in the presence of Raney Ni or with Pd/Al_2O_3 under hydrogen atmosphere

-The products of both reactions could be directly lyophilized and for the most part underwent minimal side reactions

J. Am. Chem. Soc. **2001,** *123,* 526-533

Peptide Synthesis: Process Considerations

Table 1 | **Peptide pharmaceuticals manufactured by chemical synthesis**

Peptide	Length	Manufacturing method
Adrenocorticotropic hormone (1–24)	24	C
Bivalirudin	20	C
Growth-hormone-releasing factor (1–29)	29	SP
Integrelin	7	C
Oxytocin	9	C
Atosiban (oxytocin antagonist)	9	C
Thymopentin (TP-5)	5	C
Thymosin α-1	28	SP
Thyrotropin-releasing hormone	3	C
Vasopressin analogues		
Desmopressin	9	C, SP
Felypressin	9	C
Glypressin	12	C
Lypressin	9	C
Pitressin	9	C
Corticotropin-releasing factors		
Human	41	SP
Ovine	41	SP
Angiotensin-converting enzyme inhibitors		
Enalapril, Lisinopril	2	C
Somatostatin and analogues		
Somatostatin and analogues	14	C, SP
Octreotide	8	C
Lanreotide	8	SP
Luteinizing-hormone-releasing hormone	10	C, SP
LHRH agonists and antagonists		
Leuprolide	9	C, SP
Goserelin	10	C
Triptorelin	10	C
Buserelin	9	C
Nafarelin	10	C
Cetrorelix	10	SP
Ganirelix	10	C
Calcitonins		
Human	32	C
Salmon	32	C, SP
Eel	32	C, SP
Dicarba-eel (elcatonin)	31	C, SP

C, classical solution-phase chemical synthesis; LHRH, luteinizing-hormone-releasing hormone; SP, linear solid-phase peptide synthesis.

- There are more than 40 chemically synthesized peptide therapeutics on the market today (a four-fold increase from 1990)

- All examples at left are potent hormones or hormone analogues that are required in relatively small quantities (<50 kg/ year)

- These peptides represent research undertaken at least 10 years ago and hence represent the inefficient, expensive methodology of peptide synthesis in the early 90s

- It was once thought that peptide therapeutics could be expected to cost $75-100 per gram per amino acid residue to produce

- With current advancements in chemistry and economies of scale, peptides could be produced at <$1 per gram on a multi tonne scale

-The economic advancements led to the development of enfuvirtide (a.k.a. Fuzeon or T-20)

Nat. Rev. Drug Disc., **2003,** *2,* 587-593

Peptide Synthesis: Process Considerations

Key Problems in Process Scale Peptide Synthesis

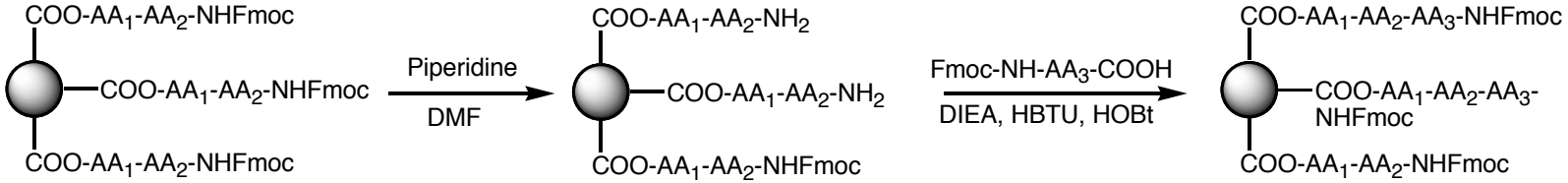
1) Contaminants and Impurities

a) Racemization: Difficult to detect in large peptides
-Use proper racemization suppresants, low dielectric constant solvents, and short activation times

b) Reaction By-Products:
- Ureas, phosphonium salts, and scavengers are the most common byproducts

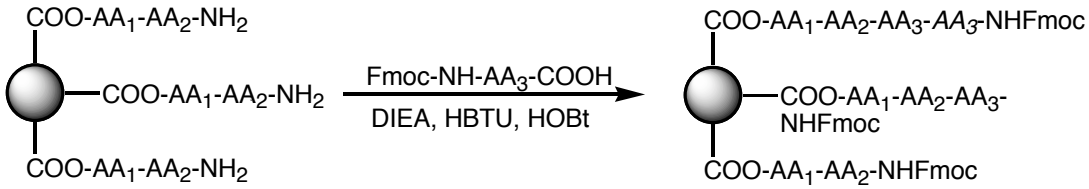
c)Oxidized peptides: Peptides rich in cysteine are easily oxidized and form dimers and higher order oligomers in solution

d) Peptide Sequence Failures
-Deletions can be minimized by using multiple methods for detection of primary amines (Ninhydrin, Trinitrobenzene Sulfonic Acid, and NF-31)



Single-Site Deletion

Nat. Rev. Drug Disc., **2003,** *2,* 587-593
Chimica Oggi. **June 2003***,* 6-11
Anal. Biochem. **1976,** *71,* 260
Eur. J. Org. Chem. **1999,** 2787



Single-Site Double Hit

Peptide Synthesis: Process Considerations

Key Problems in Process Scale Peptide Synthesis

2) Toxic and Hazardous Reagents Used in Synthesis

a) Storage and Bulk Use of Reagents

-Triazole based reagents such as HBTU can have stability problems

- This is especially pronounced with HOAt which is sensitive to friction and spark, leading to burning and explosion

- The use of such reagents may require an expensive safety upgrade

b) Hazardous Reagents

- Carbodiimides and benzotriazole reagents are known to cause skin irritation and contact dermatitis with prolonged use

- They also cause sensitization of the respiratory tract over time

3) Yield

-Ramifications of low yielding reactions are obvious, however the extent of the problem is magnified in a step wise synthesis with few purification steps

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Nat. Rev. Drug Disc., **2003,** *2,* 587-593
Chimica Oggi. **June 2003***,* 6-11

Peptide Synthesis: Enfuvirtide

- Enfuvirtide is a 36 residue peptide that selectively inhibits HIV-1 membrane fusion

-It has been approved for treatment of HIV patients in the US

- Projected requirements are 3 tonnes per year, where patients will receive 180 mg per day or 80g per year

- The drug was developed at Trimeris and a streamlined synthesis was developed by Roche on tonne scales

-This involved a hybrid solid/solution phase synthesis where small segments were first made by SPPS and then coupled in solution

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Ac-Tyr-Thr-Ser-Leu-Ile-His-Ser-Leu-Ile-Glu-Glu-Ser-Gln-Asn-Gln-Gln-Glu-Lys-Asn-Glu-Gln-Glu-Leu-Glu-Leu-Asp-Lys-Trp-Ala-Ser-Leu-Trp-Asn-Trp-Phe-NH_2

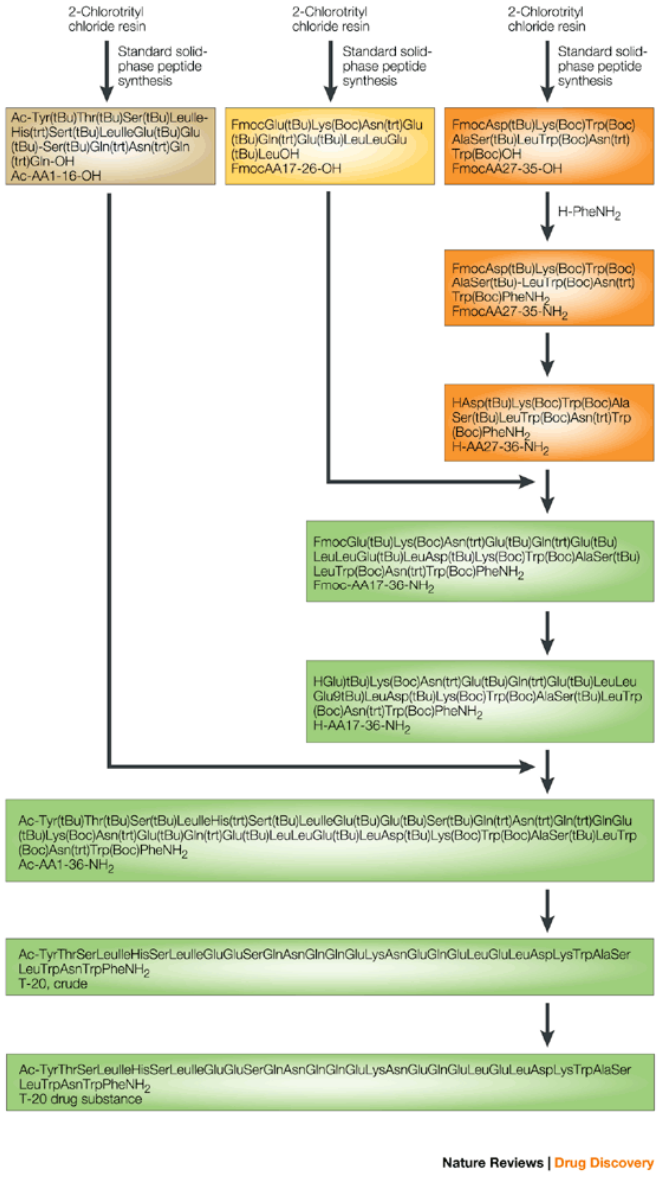
Route 1: Linear Solid Phase Peptide Synthesis

- Fmoc SPPS conducted for the 36 residue sequence
- Greater than 2 equivalents of Fmoc-AA were used per coupling
- Furthermore, upon cleavage from the resin, the peptide was only ~30-40% pure
- This required difficult, low throughput chromatographic separation
- Overall yield was 6-8%
- This was an expensive and inefficient initial synthesis, but allowed access to enough material for clinical trials

Nat. Rev. Drug Disc., **2003,** *2,* 587-593

Peptide Synthesis: Enfuvirtide

Ac-Tyr-Thr-Ser-Leu-Ile-His-Ser-Leu-Ile-Glu-Glu-Ser-Gln-Asn-Gln-Gln-Glu-Lys-Asn-Glu-Gln-Glu-Leu-Glu-Leu-Asp-Lys-Trp-Ala-Ser-Leu-Trp-Asn-Trp-Phe-NH_2



Route 2: Combination of SPPS and fragment condensations

- Three side chain protected fragments are constructed using a super acid sensitive resin, 2-chlorotrityl resin

- Resin is not patent protected and can be easily recycled, also attachment is racemization-free

- The three fragments were synthesized using HBTU/HOBt and 1.5 eq of Fmoc protected amino acids, no re-couple cycles were necessary

- Each fragment is isolated in >85% yield and >90% purity

- Each fragment can be synthesized in one week and in 300-500 kg scales

- To make the process efficient solvent recycling must occur, while yields are >99% per coupling, the cost is 75L of solvent per kilogram resin

- Five solution phase reactions complete the peptide which is then isolated in 30% overall yield

- The segment condensations were optimized to show less than 1% racemization

Nat. Rev. Drug Disc., **2003,** *2,* 587-593

Conclusions

- Amide Bonds are ubiquitous in nature and elsewhere

- Effective methods to make these bonds are critical to extending pharmaceutical and bio-organic research

- There however is no ONE method that is effective in every situation

- Unfortunately the process of selecting the proper tool is somewhat empirical, however the tool box is well stocked and most difficulties can be overcome