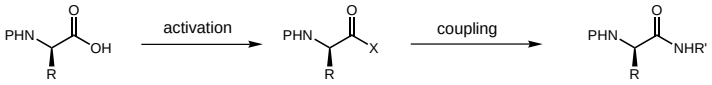
Advances in Peptide Coupling



Lead References:

K. Devries, Evans group Friday seminar, 4/9/91

L. Carpino *Methods in Enzymology* **1997**, *289*, 104

Evans Group Seminar

Jeff Katz

12/15/98

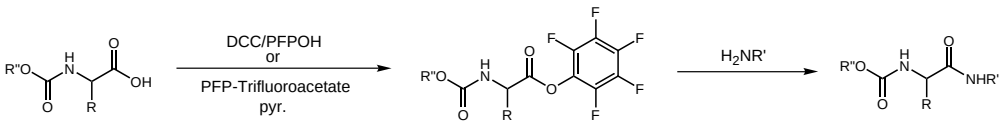
Overview

1. The role of DMAP

2. Amide formations
   - i. Carbodiimides
   - ii. Mixed anhydrides of carbon
   - iii. Acyl azides & cyanides
   - iv. Mixed anhydrides of phosphorus
   - v. Pentafluorophenyl esters
   - vi. Phosphonium & aminium reagents
   - vii. Acid fluorides
   - viii. Obscurities
3. The coupling of hindered amino acids
4. Complex peptide synthesis:
   - i. Didemnin
   - ii. Microcystin LA

Pentafluorophenyl Esters

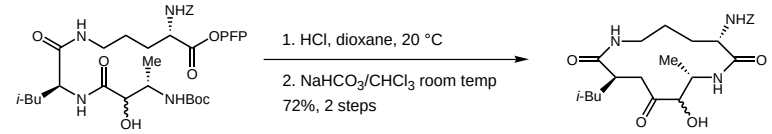
Preactivation method:

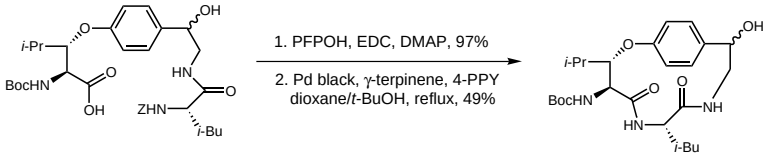


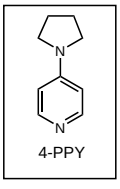
Stable, isolable active ester

Green *Tet. Lett.* **1990**, *31*, 5851

Pentaflurorophenyl Esters



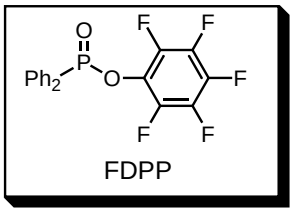


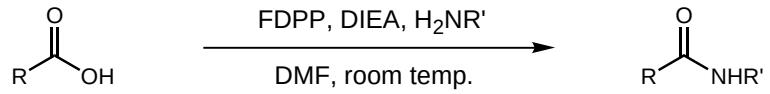


Joullie *Tet. Lett.* **1998**, *39*, 7211

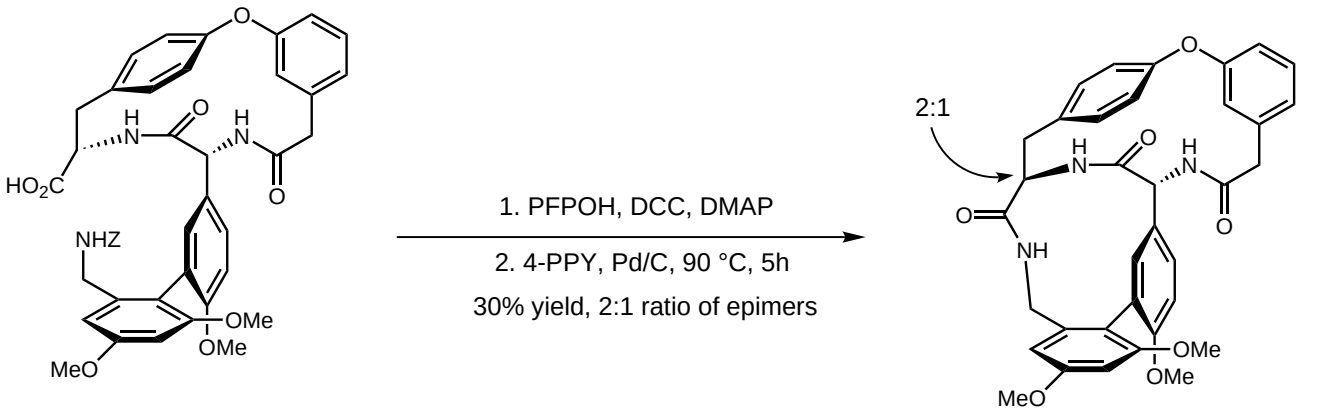
Schmidt *Chem. Commun.* **1994**, 1003

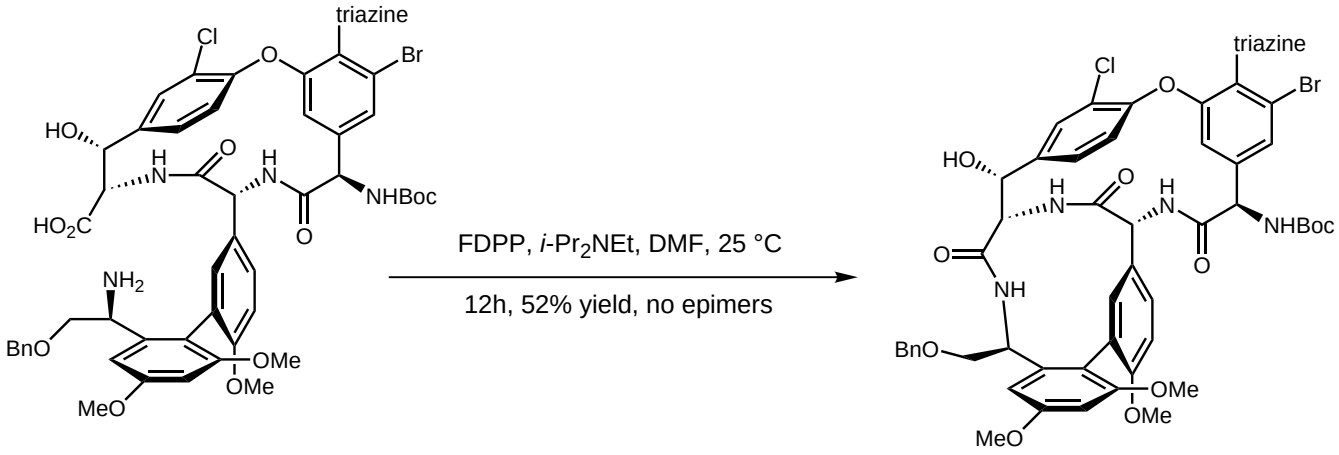
Pentafluorophenyl Esters





Xu *Tet. Lett.* **1991**, *32*, 6711

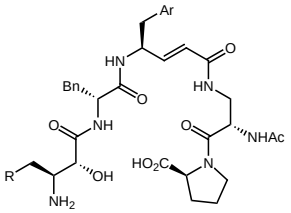




K. C. Nicolaou *Chem. Commun.* **1997**, 1899

K. C. Nicolaou *Angew. Chem. Int. Ed.* **1998**, *37*, 2708

Pentafluorophenyl Esters

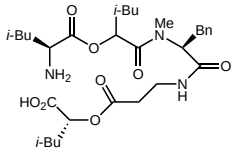


Coupling agent

Coupling agent	% Yield
EDC/HOBt	16
DPPA	8
DEPC	12
PFP ester	8
FDPP*	57

*FDPP, *i*-Pr_2NEt, DMF, r.t. 14h

Shioiri *Tet. Lett.* **1996**, *37*, 2261



Coupling agent

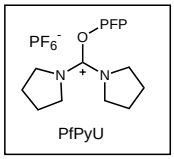
Coupling agent	% Yield
DPPA	low
FDPP	60
HATU	50
PFP ester*	85

*$CHCl_3$-$NaHCO_3$, room temp. 5h

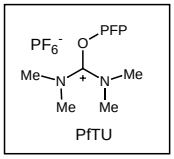
Ulrich Schmidt *Chem. Commun.* **1994**, 2381

Pentafluorophenyl Esters

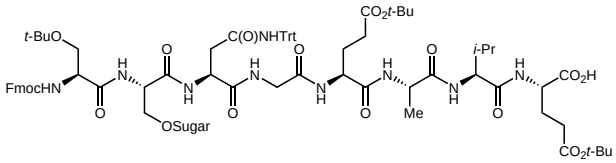
The "first" *In situ* activation method:



PfPyU



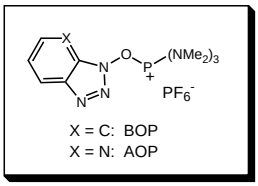
PfTU



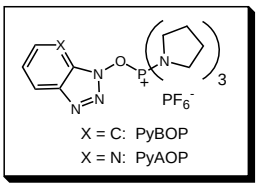
Synthesized in 13 steps on solid phase (tentagel) in an overall yield of 76% (98%/step):
(Hunig's base, collidine, PfPyU, NMP)

Habermann *J. Prakt. Chem.* **1998**, *340*, 233

Phosphonium Reagents

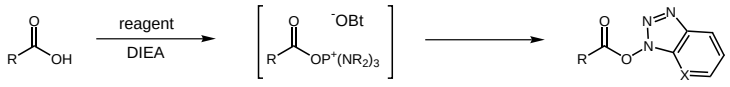


X = C: BOP
X = N: AOP



X = C: PyBOP
X = N: PyAOP

Highly active coupling reagents with "built-in" additive:

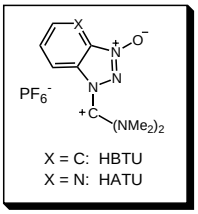


active species

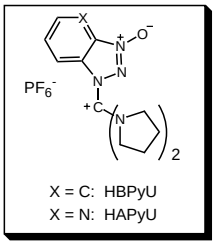
- HOAt derivatives invariably preform better than the corresponding HOBt derivatives
- Pyrollidine derivatives generally preform better than the corresponding dimethylamino derivatives

Castro *Tet. Lett.* **1975**, 1219; *Tet. Lett.* **1990**, *31*, 205
Carpino *Chem. Commun.* **1994**, 201

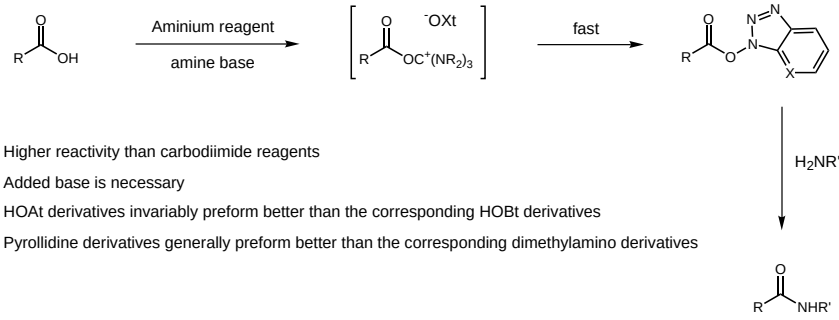
Aminium Reagents



X = C: HBTU
X = N: HATU



X = C: HBPyU
X = N: HAPyU



- Higher reactivity than carbodiimide reagents
- Added base is necessary
- HOAt derivatives invariably preform better than the corresponding HOBt derivatives
- Pyrollidine derivatives generally preform better than the corresponding dimethylamino derivatives

Knorr *Tet. Lett.* **1989**, 1927
Carpino *J. Am. Chem. Soc.* **1993**, *115*, 4397

Aminium Salt Couplings — Effects of the Amine Base

Aminium Salt	DIEA	NMM	Pyridine	collidine	lutidine	DMAP
HBPyU	10.7	5.7	7.7	0.6		
HATU		2.7		0.1		
HAPyU	2.3	1.7	6.2	0.1	0.6	10.4
HBPyU	26.6			13.7		
HATU	6.5			1.7		
HAPyU	6.3	5.9		0.1	2.3	

% Racemization for 2+1 segment coupling of Z-Phe-Val-Ala-OMe in DMF

% Racemization for 2+1 segment coupling of Z-Phe-Val-Pro-O*t*-Bu in DMF

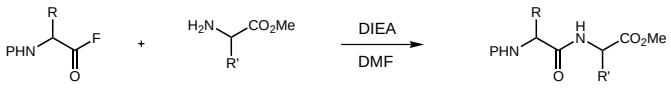
Aminium Salt	Additive	NMM (2 equiv)
HAPyU	none	12.2
HAPyU	HOAt	3.3

% Racemization for 2+1 segment coupling of Z-Phe-Val-Pro-NH_2 in DMF

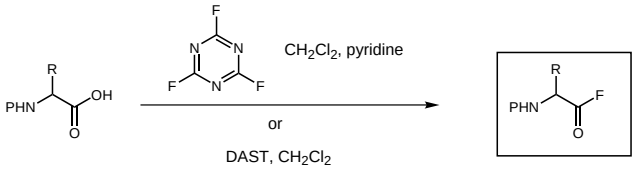
- Added HOAt/HOBt is shown to have a deliterious effect for solid phase coupling reactions

Carpino *J. Org. Chem.* **1994**, *59*, 695
Carpino *J. Org. Chem.* **1996**, *61*, 2460

Amino Acid Fluorides



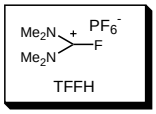
Acid Fluoride Formation:

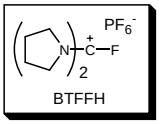


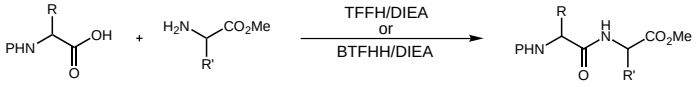
- Bench stable compounds for most Fmoc-protected amino acids
- Do not form oxazolone intermediates in the presence of tertiary amines
- Relatively unreactive to neutral oxygen nucleophiles
- React readily with anionic oxygen nucleophiles and neutral amines
- Sillicon protecting groups are problematic

Carpino *Acc. Chem. Res.* **1996**, *29*, 268

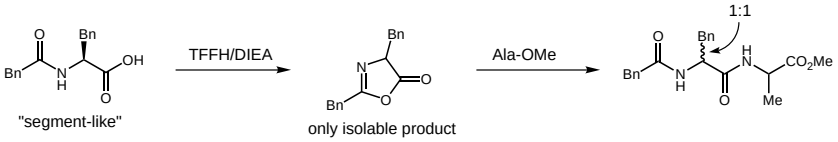
Amino Acid Fluorides — *In Situ* Generation







For segment condensations, HOAt is needed to suppress racemization:

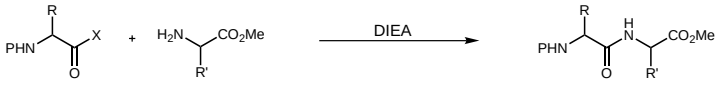


% Racemization:

	i-Pr_2NEt	NMM	collidine
TFFH	25	23	6
TFFH/HOAt	2		<0.1

Carpino *J. Am. Chem. Soc.* **1995**, *117*, 540
Carpino *Chem. Lett.* **1998**, 671

Amino Acid Fluorides



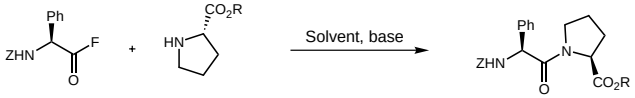
X	equiv of DIEA	% Conversion
F	0	100*
F	1	100
Cl	0	50
Cl	1	100

* Reaction rate is only slightly retarded

- No-base approach has been shown to be general for both solid- and solution-phase couplings
- No epimerization is observed

Carpino *Chem. Commun.* **1995**, 669

Acid Fluorides — Racemizaton



Solvent	base	% epimer
DMF	DIEA	19.0
DMF	collidine	11.6
CH_2Cl_2	collidine	<0.1

- Highly racemization-prone amino acids still require carefully controlled conditions

Wenschuh, Carpino *J. Org. Chem.* **1995**, *60*, 405

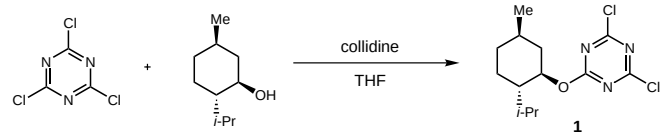
Segment Couplings

Z-Phe-Val-Pro-NH_2 via 2+1 segment coupling in DMF

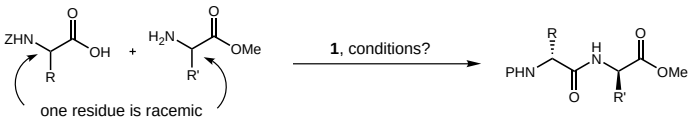
Reagent	Base (equiv)	% Yield	% epimer	Reagent	Base (equiv)	% Yield	% epimer
EDC/HOAt		**85**	**4.7**	HATU/HOAt	DIEA (2)	76	10.9
EDC/HOBt		**87**	**18.9**	HATU/HOAt	DIEA (3)	88	15.8
EDC/HODhbt		89	7.3	**HATU/HOAt**	**TMP (2)**	**72**	**2.4**
EDC•HCl/HOAt	TMP (1)	89	5.3	HATU/HOAt	TMP (3)	87	4.5
EDC•HCl/HOBt	TMP (1)	87	19.9	**HAPyU**	**TMP (2)**	**87**	**3.5**
HATU	**TMP (2)**	**83**	**5.3**	**HAPyU/HOAt**	**TMP (2)**	**76**	**1.6**
HATU	**DIEA (2)**	**86**	**13.9**	HAPyU/HOAt	TMP (3)	89	2.3
HBTU	TMP (2)	81	14.2	HAPyU/HOAt	DIEA (2)	77	3.2
BOP	TMP (2)	81	13.9	HAPyU/HOAt	DIEA (3)	90	12.1

Carpino *J. Org. Chem.* **1995**, *60*, 3561

Obscurities — Couplings with kinetic resolution



1

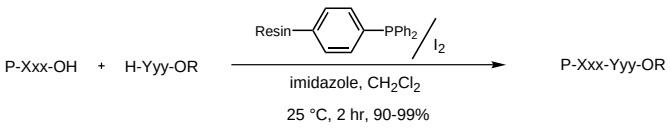


Coupling partners (substrate ratio)	% Yield	ee or de
Z-Gly-OH + D,L-Ala-OEt (1:2)	87	93 (L)
Z-D,L-Ala-OH + Gly-OEt (2:1)	86	100 (L)
Z-D,L-Ala-OH + L-Leu-OMe (2:1)	72	100 (L,L)
Z-D,L-Ala-OH + L-Phe-OEt (2:1)	75	100 (L,L)

• %ee measured by rotation only

Kaminski *Synthetic Communications* **1998**, *28*, 2689

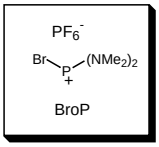
Obscurities — Polymer-bound coupling reagents



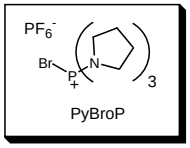
P	Xxx	Yyy	R	% Yield
Fmoc	Ala	Leu	Allyl	99
Cbz	Met	Ala	Me	98
Boc	Leu	Phe	*t*-Bu	95
Fmoc	Val	Val	Allyl	99
Fmoc	Cys(Trt)	Cys(Trt)	Me	95
Boc	Trp	Leu	Me	94

• Authors propose an acyl iodide as the active species

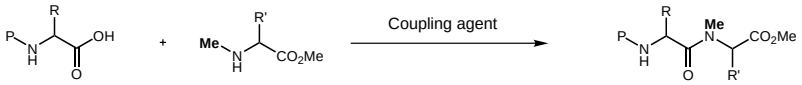
Caputo et. al. *Synthesis* **1995**, 141



Secondary Amine Coupling



More Powerful activation is needed for Secondary Amines:

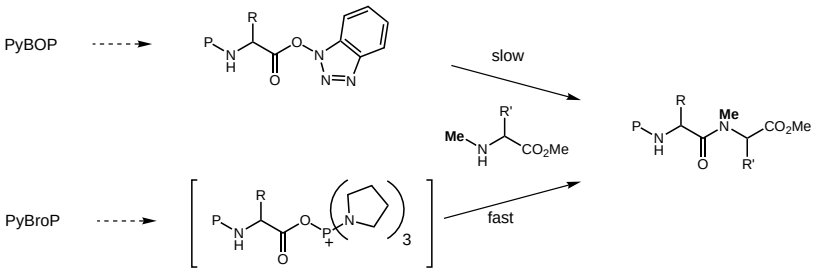


Sequence	% Yield (hours) PyBOP	PyBroP
Z-**Me**Val-Val-OMe	90 (1)	85 (1)
Z-Val-**Me**Val-OMe	11 (1)	70 (1)
Fmoc-Val-**Me**Val-OMe	30 (24)	84 (3)
Boc-Val-**Me**Val-OMe	45 (24)	44 (3)

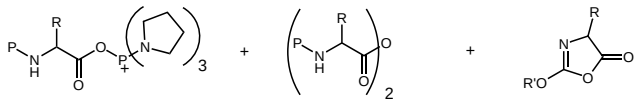
CH_2Cl_2, DIEA, room temp.

Coste *J. Org. Chem.* **1994**, *59*, 2437

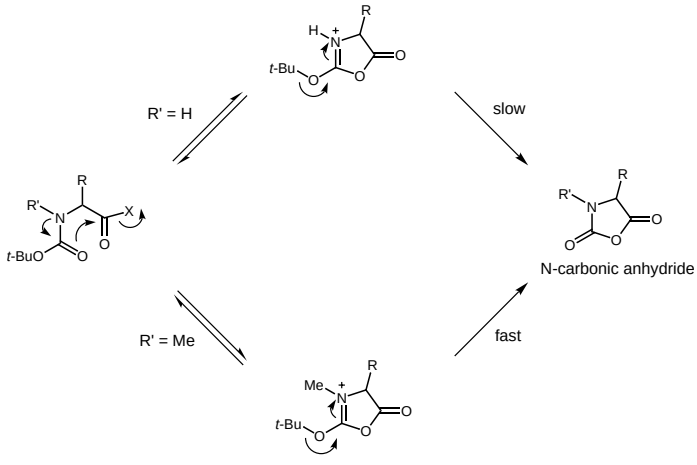
Phosphonium Reagents — Active Coupling Species



For PyBroP, The "active ester" is a mixture of species:



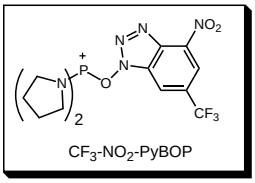
Secondary Amine Coupling: Side-product Pathways



Coste *Tet. Lett.* **1992**, *33*, 2815

Secondary Amine Couplings

An electron-poor HOBt additive can increase reactivity:



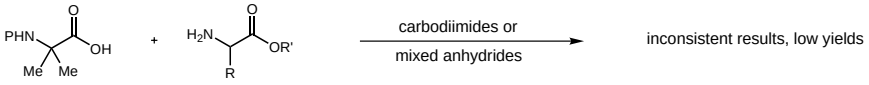
CF_3-NO_2-PyBOP

Sequence	% Yield PyBroP	% Yield CF_3-NO_2-PyBOP
Z-Val-Val-OMe	89	98
Z-Val-**Me**Val-OMe	49	76
Fmoc-Val-**Me**Val-OMe	57	85
Boc-Val-**Me**Val-OMe	25	62
Boc-**Me**Leu-**Me**Leu-OMe	47	87
Z-**Me**Val-**Me**Val-OMe	22	71

CH_2Cl_2, DIEA, room temp., 1 hr

Bloemhoff *Tet. Lett.*, **1995**, *36*, 4643

α,α-Dialkylamino Acids



Aminoisobutyric acid (Aib)

% Yield for Phosphonium-based Aib couplings (CH_2Cl_2, room temp.)

Sequence	BOP	PyBOP	BroP	PyBroP
Z-Aib-Gly-OEt	92	87	89	87
Z-Aib-Val-OMe	88		87	84
Z-Aib-Pro-O*t*-Bu	82		84	95
Z-Aib-Aib-OMe		89		77
Boc-Aib-Aib-OMe	80	86	25 76*	25 77*

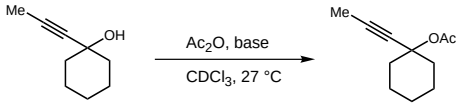
* With added DMAP

• Aib-Aib coupling is still sluggish — reaction times are 16 to 24 hours

• While good yields can be obtained, sluggish and varied reaction conditions make this unsuitable for solid phase

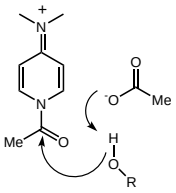
Coste *Tetrahedron* **1991**, *47*, 259

DMAP Acylation Catalysis



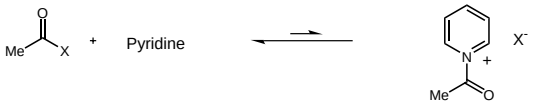
Base	Acylation Reagent	t 1/2 (min)
DMAP	Ac_2O	7
DMAP	AcCl	20
pyridine	Ac_2O	1000
1:1 pyridine:NEt_3	Ac_2O	1000

General Base Acceleration:

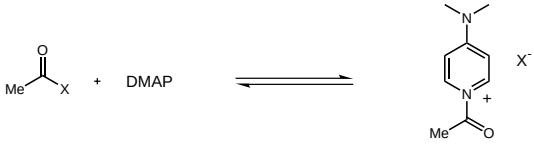


Steglich *ACIEE* **1978**, *17*, 569

DMAP — Equilibrium Concentrations



• Undetectable in non-polar solvents, but has been detected in H_2O by UV



X	% Pyridinium in solution
Cl	>95
OAc	5-10

$CDCl_3$, room temperature

α,α-Dialkylamino Acids

Aminium salts and acid fluorides for solid-phase α,α-dialkyl and *N*-methylamino acid synthesis:

H-Tyr-**Aib**-**Aib**-Phe-Leu-NH_2

Reagent	sequence purity
HATU	94%
TFFH	92%
HBTU	43%

7 min. activation, 30 min. coupling in DMF

H-D-Ala-**Me**Leu-**Me**Leu-**Me**Val-Phe-Val-OH

Reagent	sequence purity
HATU	85%
HBTU	8%

7 min. activation, 30 min. coupling in DMF

Carpino *Chem. Commun.* **1994**, 201

Peptaibols

- Bioactive linear peptide sequences
- Sequences are approx. 20 residues and contain unusually high amounts of α,α-dialkylamino acids
- Aib-Pro linkages are acid-sensitive (cleaved by TFA)

Ac-**Aib**-Pro-**Aib**-Ala-**Aib**-Ala-Gln-**Aib**-Val-**Aib**-Gly-Leu-**Aib**-Pro-Val-**Aib**-**Aib**-Glu-Gln-Pheol

alamethicin F-30

Synthesized on solid phase *via* Fmoc-amino acid fluorides in 78% purity

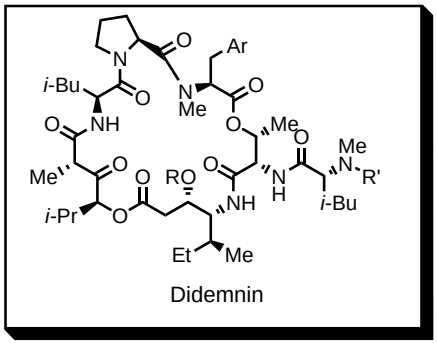
Ac-**Aib**-Ala-**Aib**-Leu-**Aib**-Gln-**Aib**-**Aib**-**Aib**-Ala-**Aib**-**Aib**-Pro-Leu-**Aib**-**Iva**-Gln-Valol

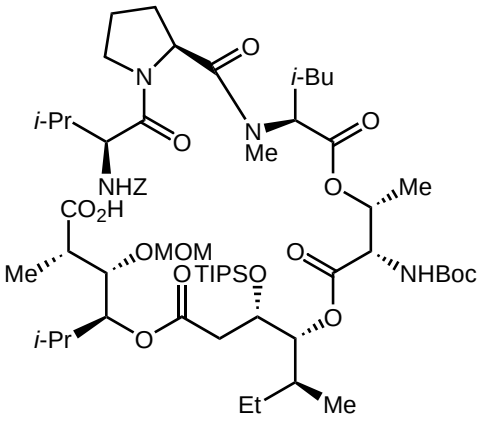
trichotoxin A-50

Synthesized on solid phase *via* Fmoc-amino acid fluorides in 60% purity

Wenschuh, Carpino *J. Org. Chem.* **1995**, *60*, 405

Didemnin





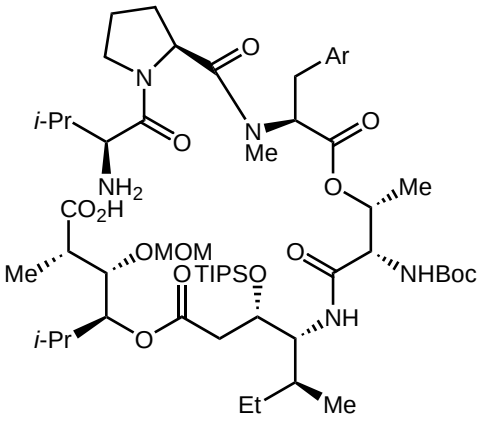
1. PFPOH, DCC, DMAP

2. Pd/C, 4-PPY, 95 °C

72%

cyclized product

• FDPP, BOP and HBTU were unsuccessful

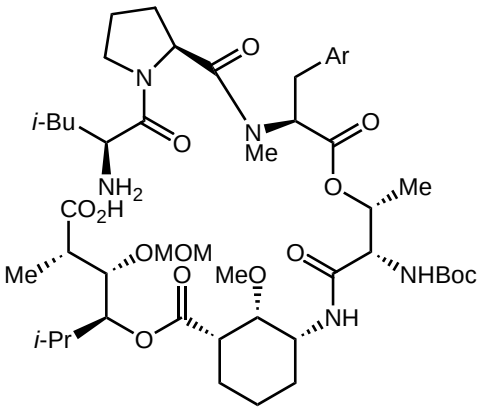


DPPA, 0 °C, 72h, 42%

or

FDPP, room temp. 4h, 68%

cyclized product



FDPP, 30%

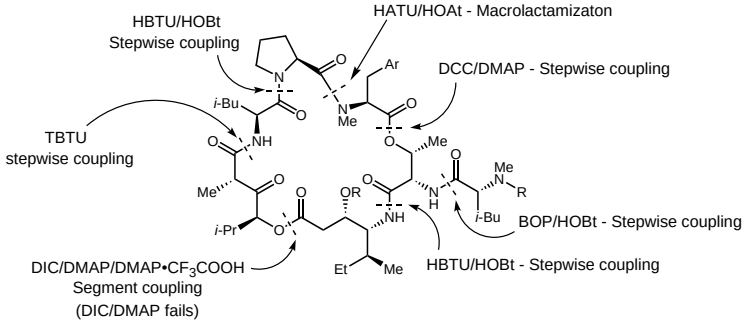
or

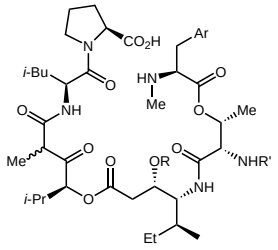
HBTU, 50%

cyclized product

Joullie *J. Org. Chem.* **1994**, *59*, 5192
Joullie *Bioorg. Med. Chem. Lett.* **1996**, *6*, 2713
Joullie *J. Org. Chem.* **1996**, *61*, 1655

Didemnin





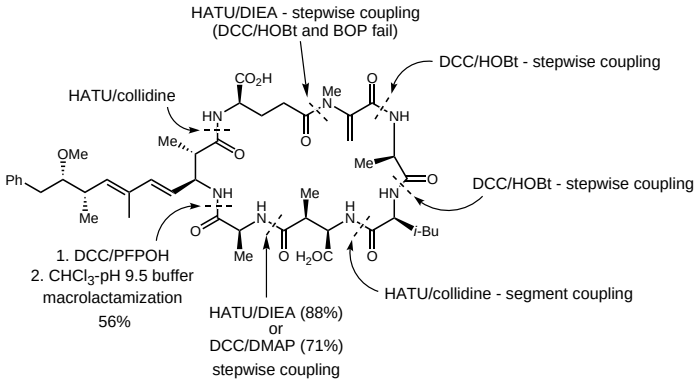
HATU, HOAt

cyclized product, 1 isomer

R = H, R' = H: 76% yield
R = TBS, R' = Boc-(*R*)-N(Me)-Leu-OH: 28% yield

Giralt *J. Org. Chem.* **1997**, *62*, 354

Microcystin LA



Richard Chamberlin *J. Am. Chem. Soc.* **1996**, *118*, 11759

Overview

Stepwise couplings:

Standard amino acids — EDC/HOBt. DEPC, or BOP are also good choices

Racemization-prone amino acids — If EDC/HOBt is insufficient, try EDC/HOAt or HATU/collidine

Segment couplings:

Phosphonium or aminium salts are good choices, HOAt/pyrollidine derivatives for the difficult cases

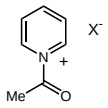
Macrolactamizations:

FDPP/PFP esters, DPPA and HAPyU/collidine are good bets

Hidered couplings:

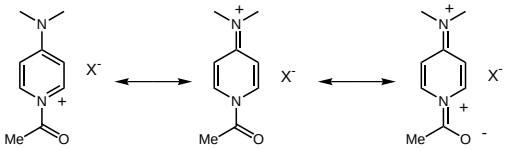
Acid fluorides and HAPyU/collidine are good choices. PyBroP and BOP-Cl work for solution phase.

DMAP Acylation Catalysis



A1 (X=Cl)

A2 (X=OAc)



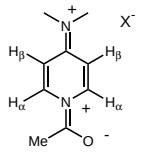
B1 (X=Cl)

B2 (X=OAc)

B'

B"

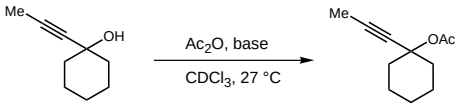
B1 and **B2** are observable by ^{1}H NMR in $CDCl_3$ or CD_2Cl_2 at low temperature



B"

4 distinct resonances

DMAP Acylation Catalysis

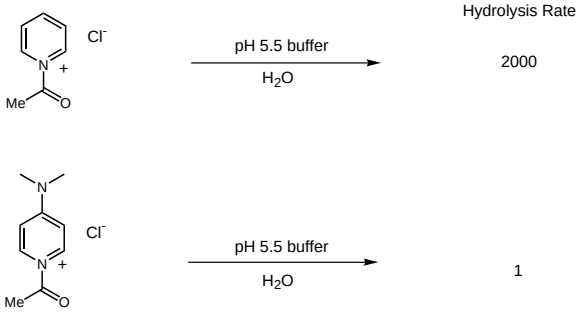


Base	Acylation Reagent	t 1/2 (min)
DMAP	Ac_2O	7
DMAP	AcCl	20
pyridine	Ac_2O	1000
1:1 pyridine:NEt_3	Ac_2O	1000

- Rate of pyridinium-acylations is tied to:
  - Concentration/solubility
  - Identity of counterion
  - **Tightness of ion pair**

DMAP Acylation Catalysis

A Reactivity Reversal:

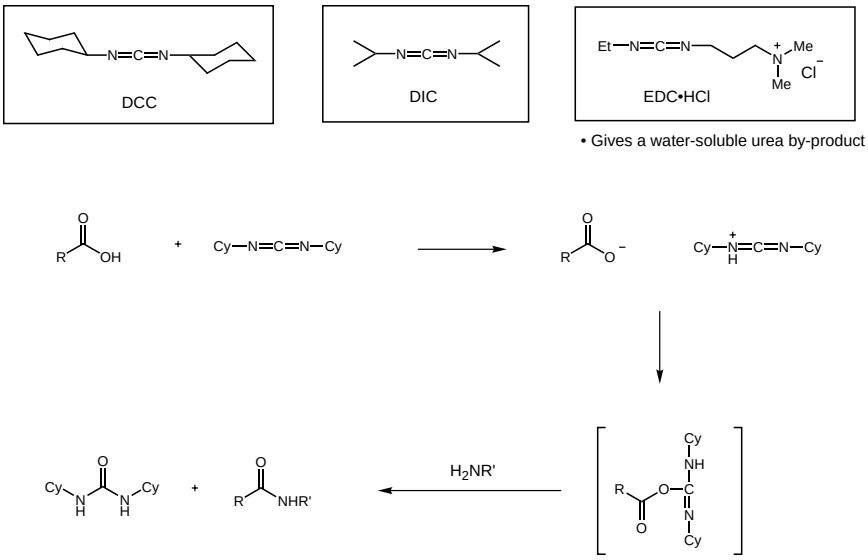


- Rate of pyridinium-acylations is tied to:
  - Tightness of ion pair
  - Concentration/solubility
  - Identity of counterion

Overview

1. The role of DMAP
2. **Amide formations**
   - i. Carbodiimides
   - ii. Mixed anhydrides of carbon
   - iii. Acyl azides & cyanides
   - iv. Mixed anhydrides of phosphorus
   - v. Pentafluorophenyl esters
   - vi. Phosphonium & aminium reagents
   - vii. Acid fluorides
   - viii. Obscurities
3. The coupling of hindered amino acids
4. Complex peptide synthesis:
   - i. Didemnin
   - ii. Microcystin LA

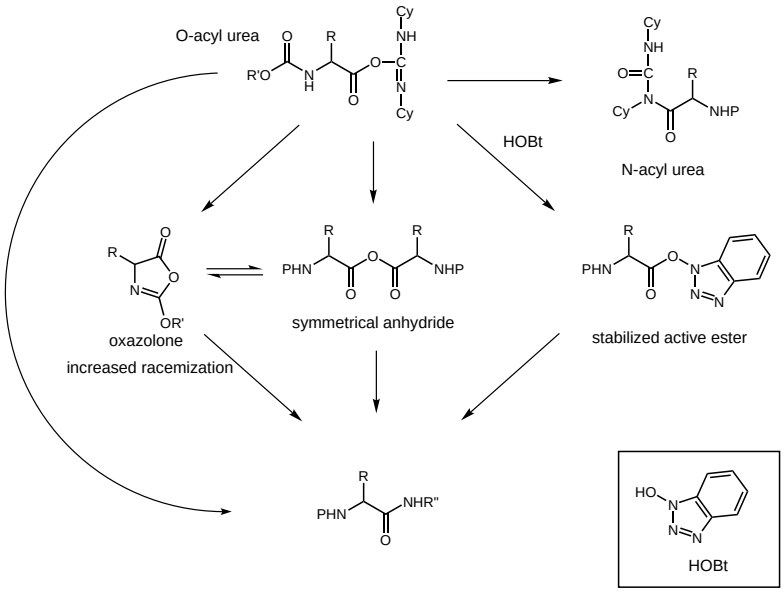
Carbodiimide Activation



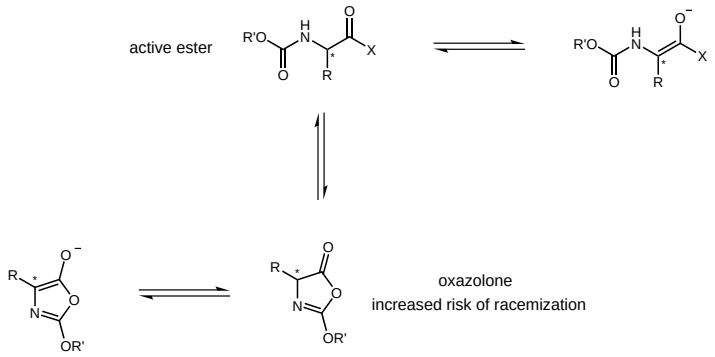
Sheehan *J. Am. Chem. Soc.* **1955**, *77*, 1067

Rebek *J. Am. Chem. Soc.* **1973,** *95*, 4052

Carbodiimides — Reaction Pathways



Racemization Pathways

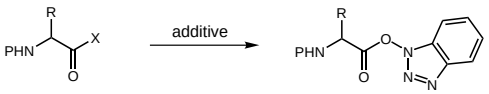


- Direct deprotonation of active esters does occur, but oxazolone formation is the major racemization pathway
- Carbamate-protected amines help supress oxazolone formation and deprotonation
- Amide "amino-protection" is the main reason for increased racemization in segment couplings

---

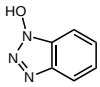
Modern Advances in Coupling Additives

Higher reactivity
Increased racemization
More side-reactions
Moisture-sensitive

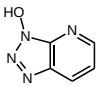


Lower reactivity
Decreased racemizaton
resistant to side-reactions
Less water sensitive

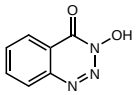
Most common coupling additives:



HOBt



HOAt

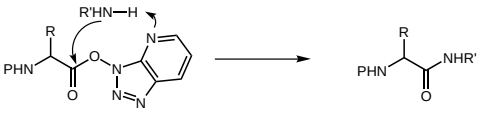


HODhbt

- Decreased racemization vs. HOBt but more side-reactions

Z-Phg-Pro-NH_2 formation in DMF:

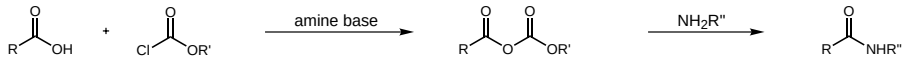
Reagent	% Yield	% epimer
EDC/HOAt	81	7
EDC/HOBt	80	25
EDC/HODhbt	82	10



- HOAt has the advantage of internal base acceleration

Konig *Chem. Ber.* **1970**, *103*, 2024,2034
Carpino *J. Org. Chem.* **1995**, *60*, 3561

Mixed Anhydrides of Carbon



Amine	equiv	% Yield	% epimer
NMe_3	1	90	0
NMe_3	2	<5	68
NEt_3	1	82	8
NEt_3	2	59	16
i-Pr_2NEt	1	3	0.2
i-Pr_2NMe	1	94	0
i-Pr_2NMe	2	85	3
NMM	1	92	0
NMM	2	93	0

Z-Gly-Phe-Gly-OEt synthesis in THF at -15 °C,
12 min activation with *i*-BuOC(O)Cl

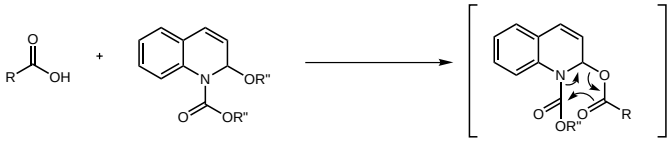
Common chloroformates

- The solvents of choice are ethyl acetate and THF. Acetonitrile, CH_2Cl_2, NMP and DMF should be avoided.
- Side reactions: disproportionation to symetrical anhydride, attack at undesired carbonyl

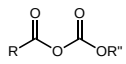
Anderson *J. Am. Chem. Soc.* **1967**, *89*, 5012
Jouin *Tetrahedron* **1989**, *45*, 5039

Mixed Anhydrides of Carbon

***In situ* generation (no preactivation) of mixed anhydrides:**



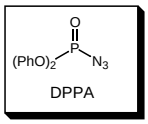
EEDQ: R" = Et
IIDQ: R" = *i*-Bu



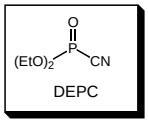
Yajima *Chem. Commun.* **1972**, 942
Belleau *J. Am. Chem. Soc.* **1968**, *90*, 1651

- No additional base is necessary

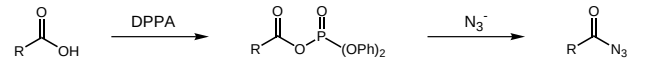
DPPA and DEPC



• Tends to be used for macrocylizations



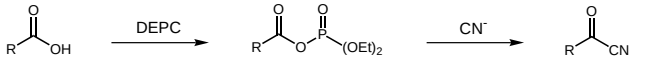
• Tends to be used for stepwise couplings



active species

• Curtius rearrangement is slow relative to coupling

• Acyl azide relatively unreactive to non-amine nucleophiles

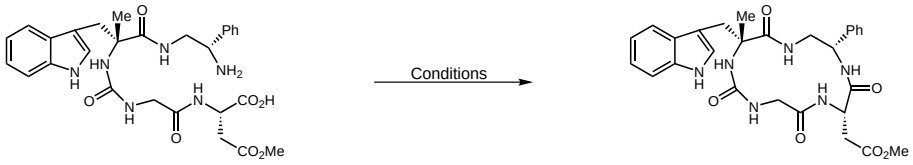


active species

Yamada *J. Am. Chem. Soc.* **1972**, *94*, 6203

Yamada *Tet. Lett.* **1973**, 1595

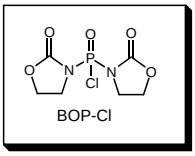
DPPA

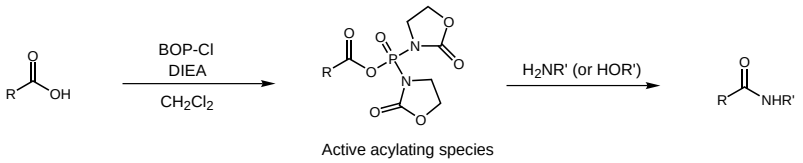


Method	% Yield
DPPA	71
BOP	49
DCC, HOBt	31

Pritchard *Tetrahedron* **1992**, *48*, 8471

BOP-Cl Activation

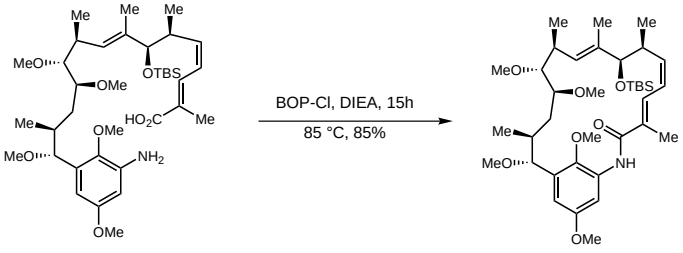




- Mixed anhydride intermediate is very reactive and will form both esters and amides
- Less prone to side-reactions than chloroformate-based anhyrides
- Active enough to couple N-alkyl amino acids, but requires long reaction times

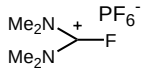
A. Palomo-Coll *Synthesis* **1980**, 547
Daniel Rich *J. Am. Chem. Soc.* **1985**, *107*, 4342
Daniel Rich *J. Org. Chem.* **1986**, *51*, 3350

BOP-Cl Macrolactamization

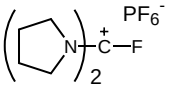


DCC, DEPC, DPPA fail

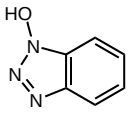
Baker *Chem. Commun.* **1989**, 378



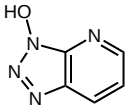
TFFH



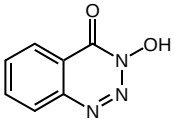
BTFFH



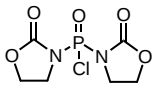
HOBt



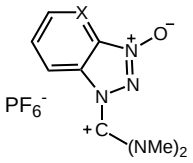
HOAt



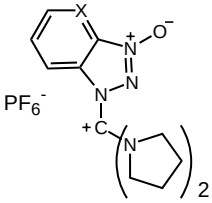
HODhbt



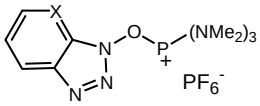
BOP-Cl



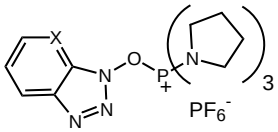
X = C: HBTU
X = N: HATU



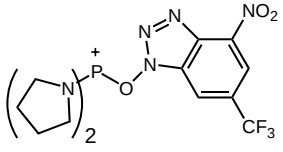
X = C: HBPyU
X = N: HAPyU



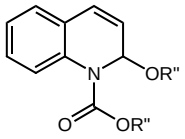
X = C: BOP
X = N: AOP



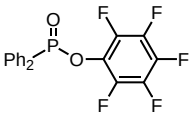
X = C: PyBOP
X = N: PyAOP



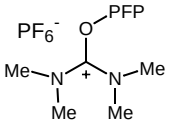
CF_3-NO_2-PyBOP



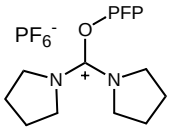
EEDQ: R" = Et
IIDQ: R" = *i*-Bu



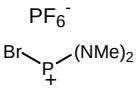
FDPP



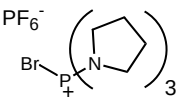
PfTU



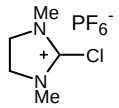
PfPyU



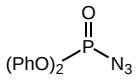
BroP



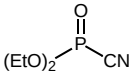
PyBroP



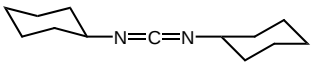
CIP



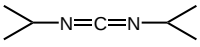
DPPA



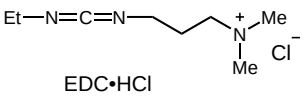
DEPC



DCC



DIC



EDC•HCl