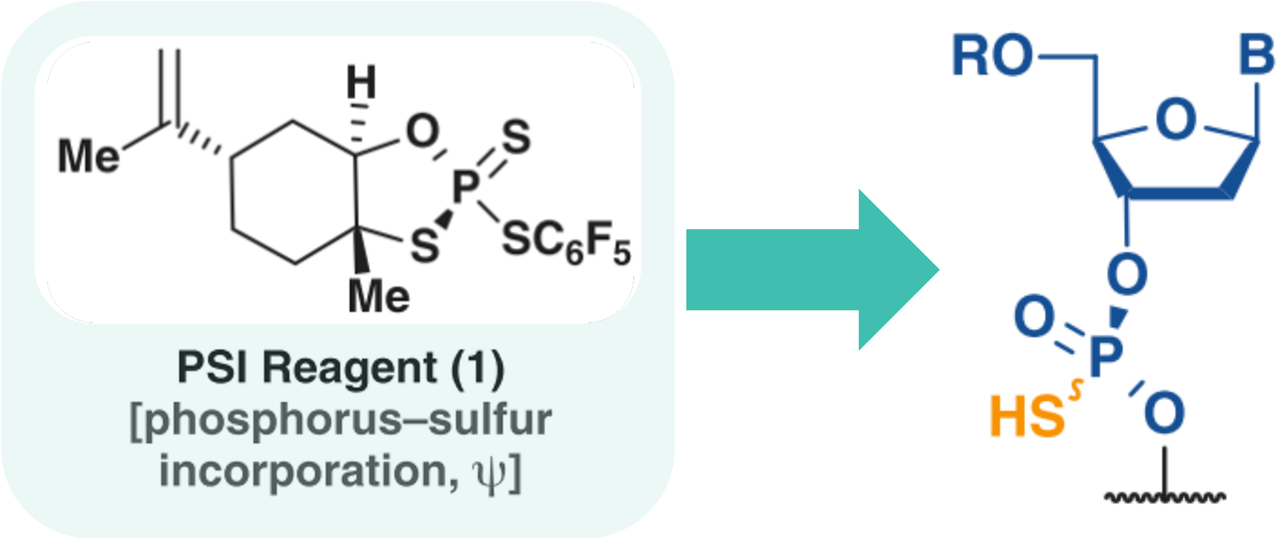
P(V) reagents for stereodivergent synthesis of phosphothioate



Literature Seminar　M2 Fujiyoshi　2021/7/29

Knouse, K. W.; Baran, P.; *et al. Science*, **2018**, *361*, 1234

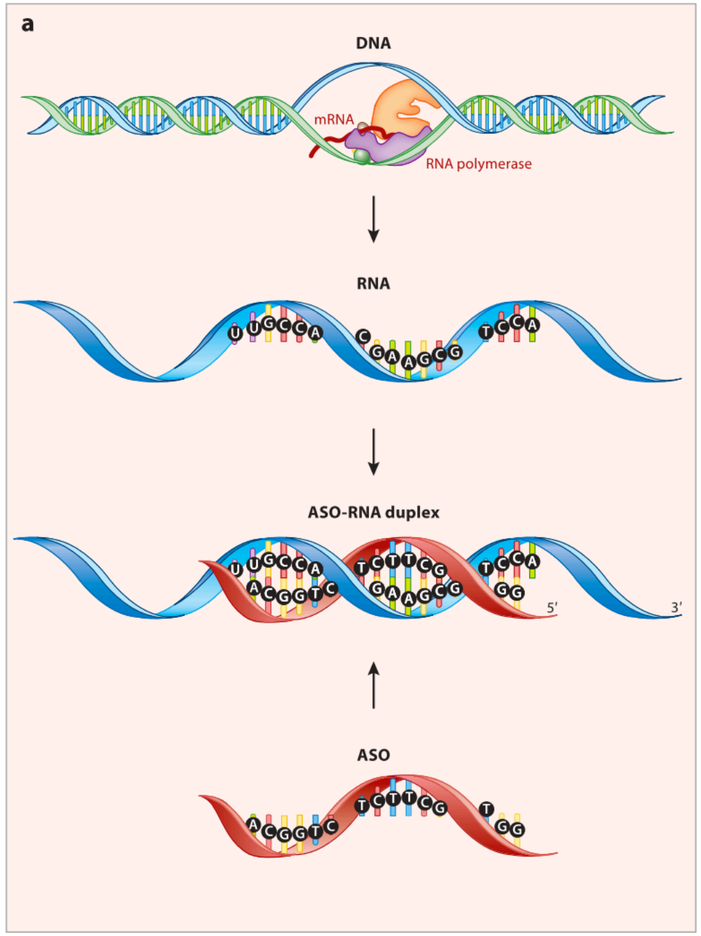
Contents

- Introduction of antisense oligonucleotide
- Reagents for chiral phosphorothioate synthesis: ψ reagent
- The principle of ψ reagent's reactivity
  - Stereoselectivity
  - Chemoselectivity
  - High reactivity
- Summary

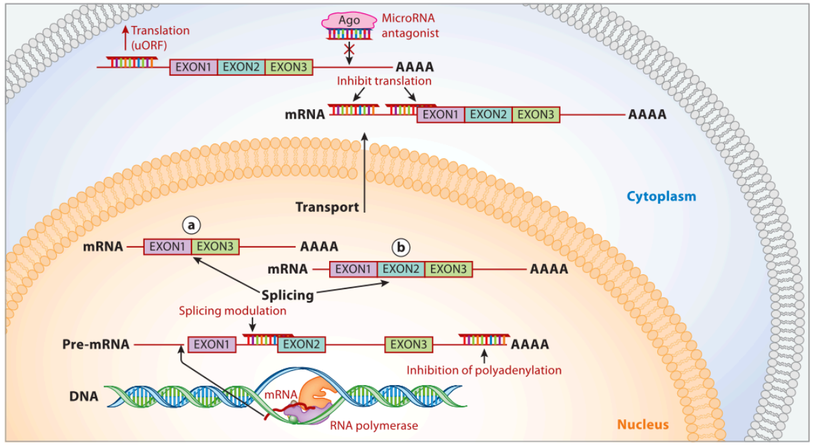
Contents

Antisense Oligonucleotide (ASO)

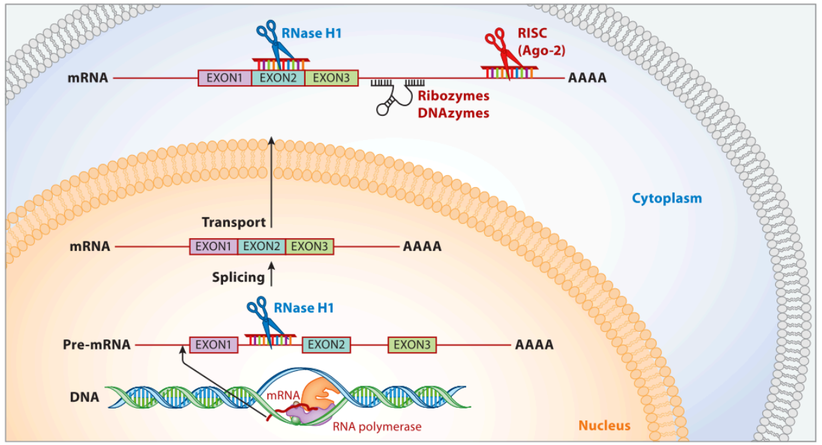
ASO binds to RNA



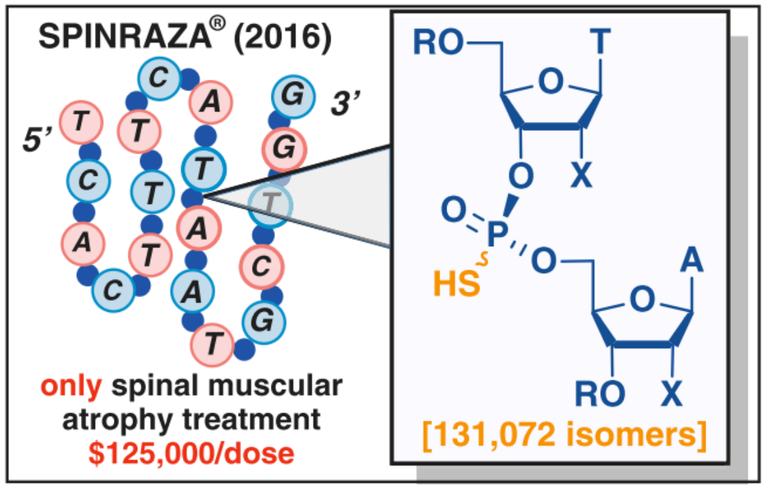
Steric block mechanism



RNA degradation mechanism



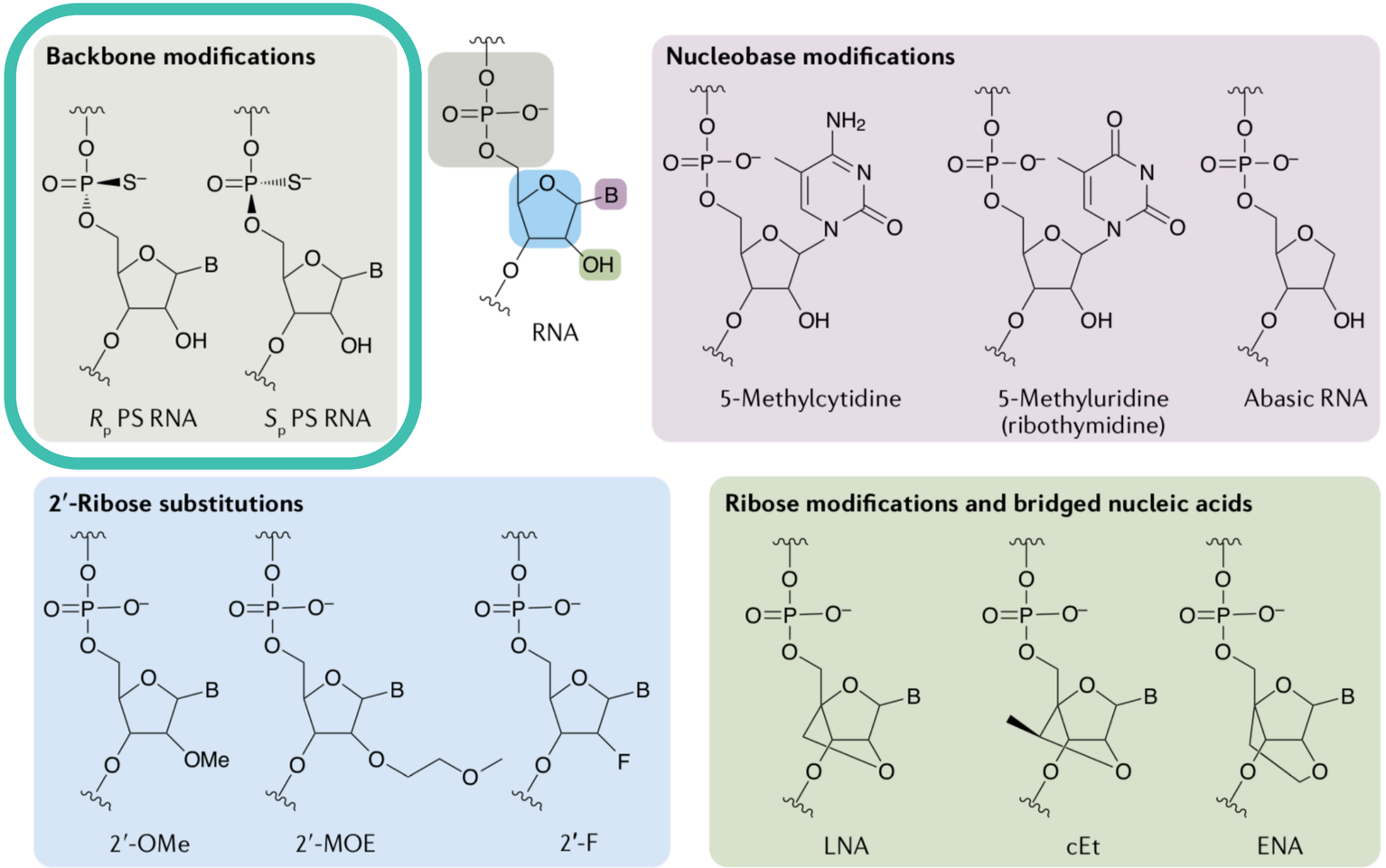
One of the FDA approved ASOs



Knouse, K. W.; Baran, P.; *et al. Science*, **2018**, *361*, 1234

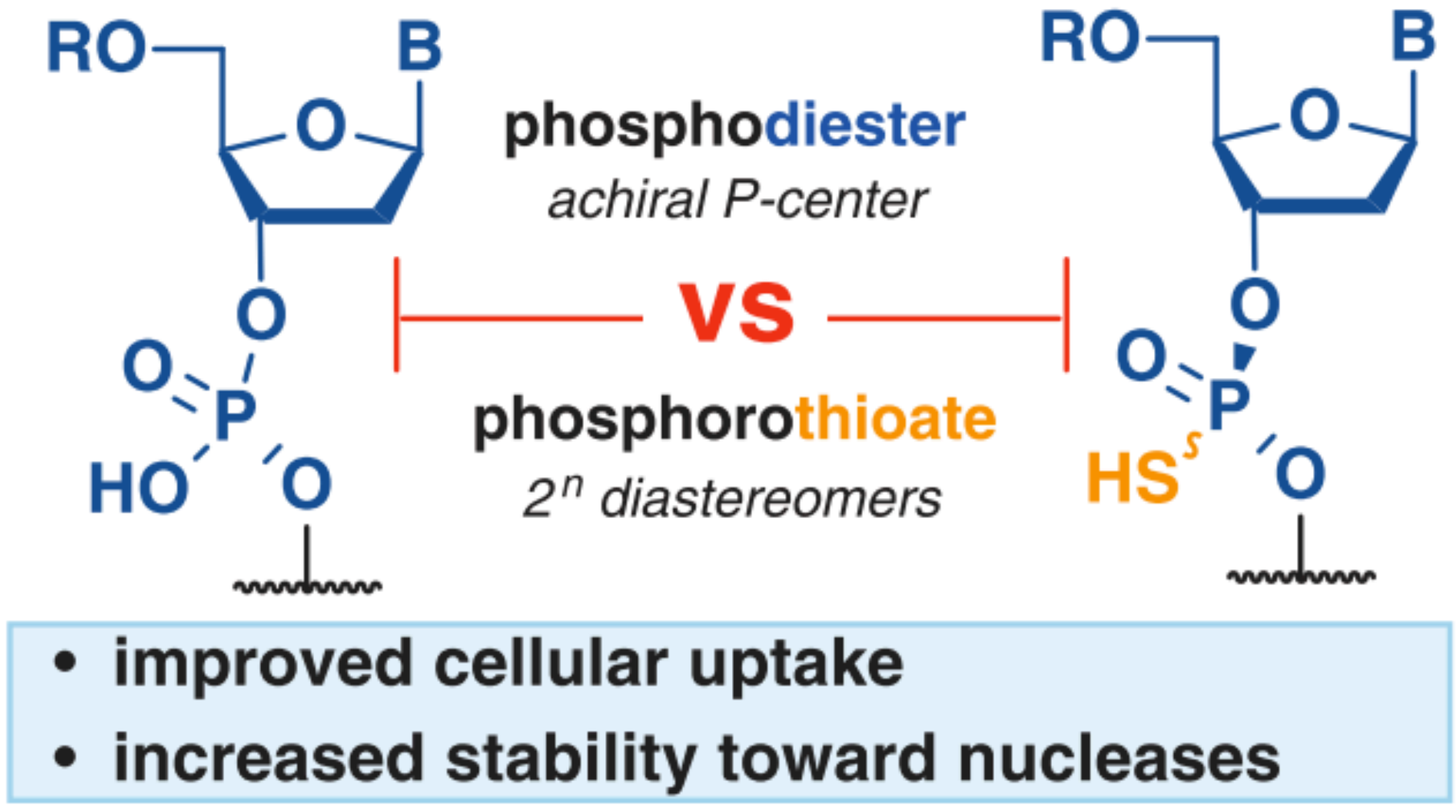
Bennett, C. *Annu. Rev. Med.*, **2019**, *70*, 307

Common chemical modifications in ASO



T. Roverts *et al.*, *Snat. Rev. Drug. Discov.*, **2020**, *19*, 673

Why Sulfur? The Thio effect



Knouse, K. W.; Baran, P.; *et al. Science*, **2018**, *361*, 1234

(See an latest article for mechanism of cellular uptake
Laurent, Q.; *et al. Angew. Chem. Int. Ed.* **2021**, *60*, 1)

The stereochemistry at phosphorus

The **stereochemistry** of phosphorothioate affect

- the stability against nucleases
- the efficiency of degradation of the targeted RNA.

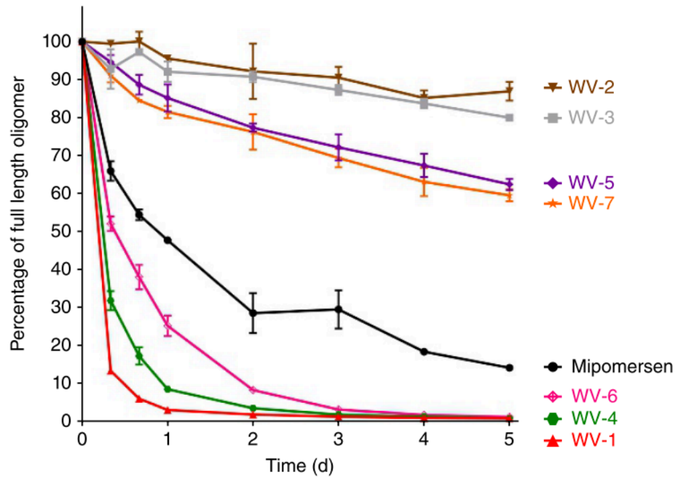
Mipomersen and stereochemically pure counterparts

ASO	Sequence	Number of stereoisomer	Tm (°C)	V0 (μM/min)
Mipomersen	GCCTCAGTCTGCTTCGCACC	524,288	80.2	0.05
WV-1	GCCTCAGTCTGCTTCGCACC	1	84.7	---
WV-2	GCCTCAGTCTGCTTCGCACC	1	74.7	---
WV-3	GCCTCAGTCTGCTTCGCACC	1	78.8	---
WV-4	GCCTCAGTCTGCTTCGCACC	1	80.0	---
WV-5	GCCTCAGTCTGCTTCGCACC	1	81.6	0.13
WV-6	GCCTCAGTCTGCTTCGCACC	1	78.3	---
WV-7	GCCTCAGTCTGCTTCGCACC	1	68.2	---

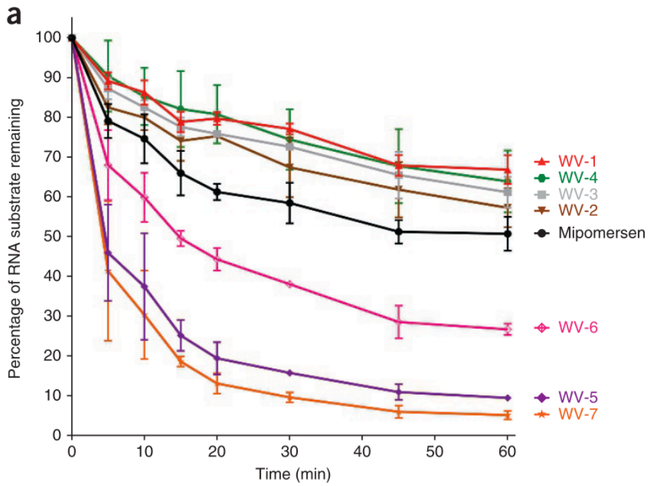
◇ Stereorandom ^ *Rp* v *Sp*

○ DNA ○ 5-Methyl DNA ○ 2′-Methoxyethyl (MOE) ○ 5-Methyl 2′-Methoxyethyl (MOE)

Stability in rat liver homogenate



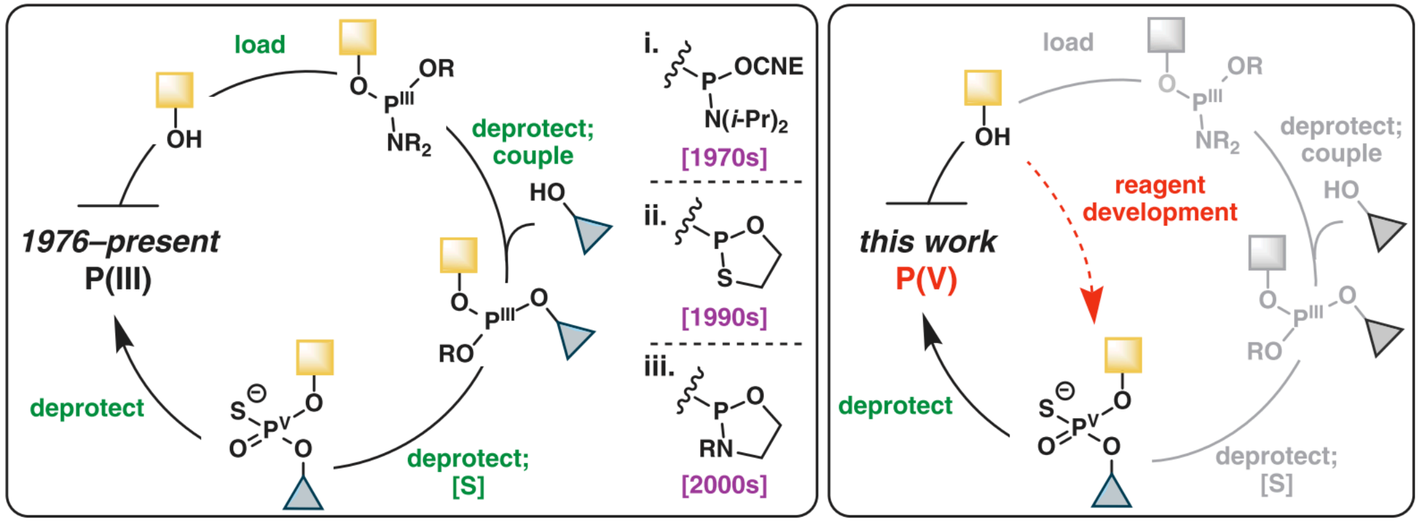
Activity of RNase HC on ASO–RNA heteroduplexes



N. Iwamoto *et al.*, *Nat. Biotechnol.*, **2017**, *35*, 845

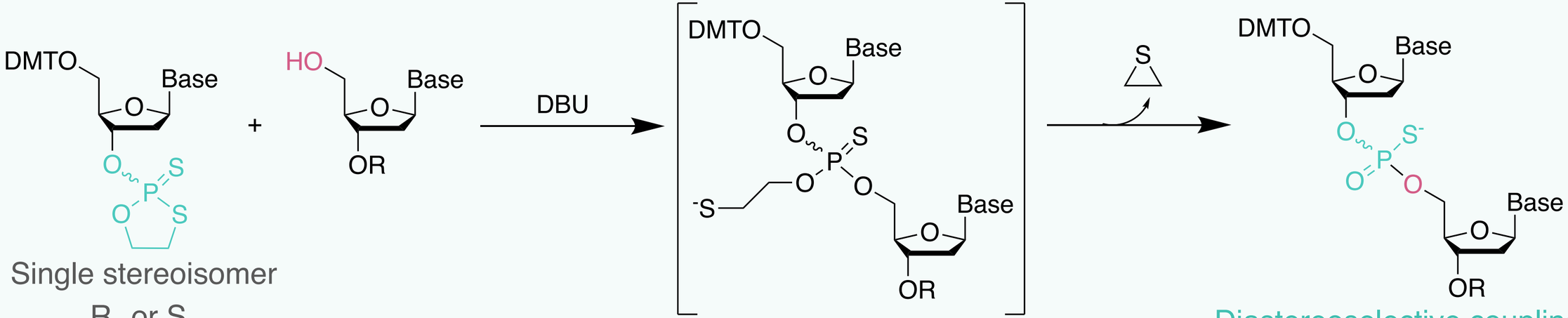
What is problems of current methods for construction of PS linkage?

- Stepwise-synthesis using P(III) chemistry is operationally cumbersome.
- P(III) reagent is air and moisture sensitive.
- The stereochemistry at phosphorus is often disregarded in scalable preparation.



Knouse, K. W.; Baran, P.; *et al. Science*, **2018**, *361*, 1234

5’-O-DMT-nucleoside 3’-O-(2-thio-1,3,2-oxathiaphospholane)

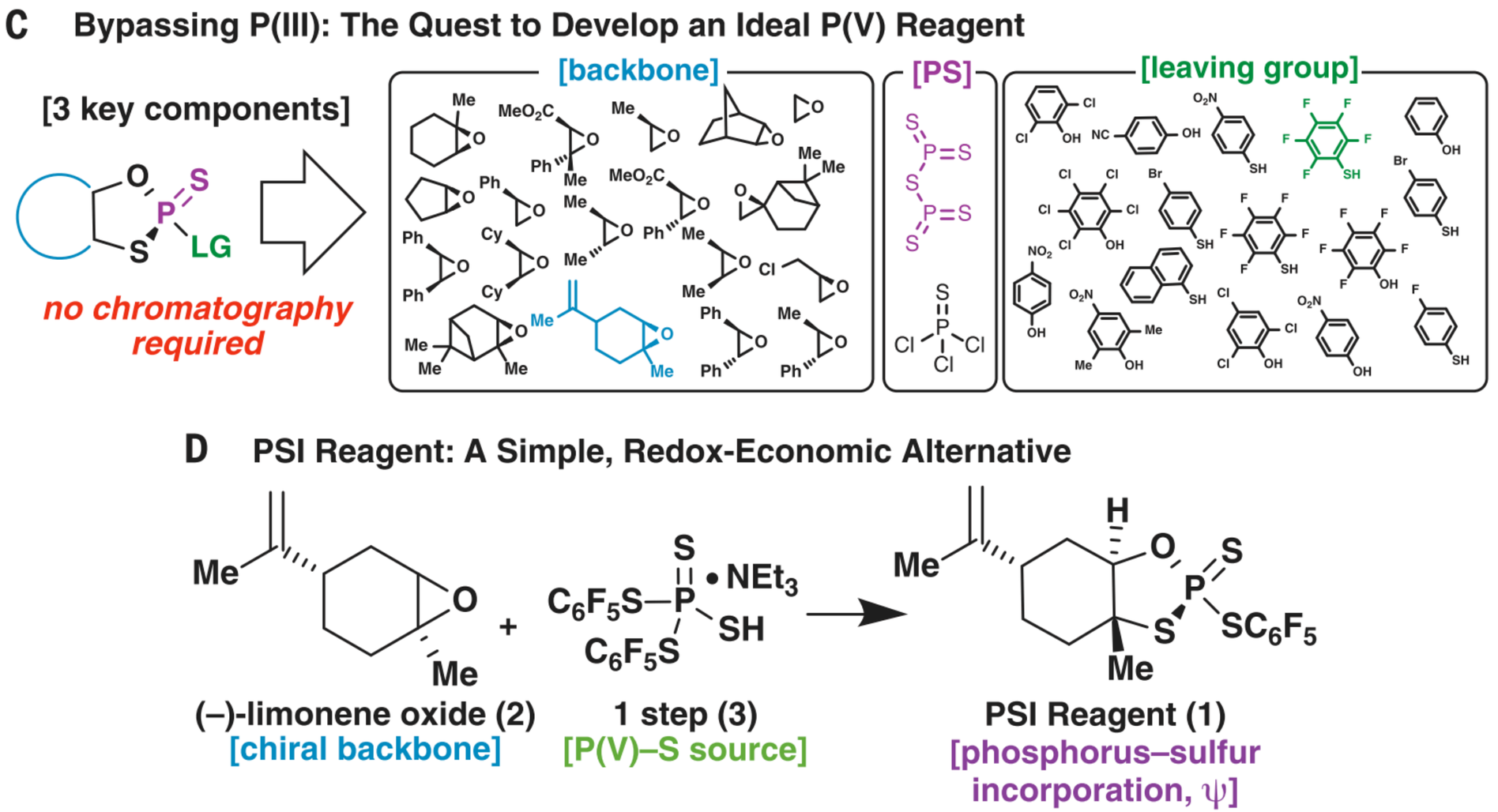


Problems

- 3(4) step synthesis of nucleotide loaded reagent **via phosphoramidite P(III)** (See appendix)
- **Separation of the diastereomers of reagents**
- **Scale limited**

Stec, W.; *et al. J. Am. Chem. Soc.* **1995**, *117*, 863
Knouse, K. W.; Baran, P.; *et al. Science*, **2018**, *361*, 1234

Phosphorus–Sulfur Incorporated (PSI, ψ) reagent



Knouse, K. W.; Baran, P.; *et al. Science*, **2018**, *361*, 1234

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- Introduction of antisense oligonucleotide
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  - Chemoselectivity
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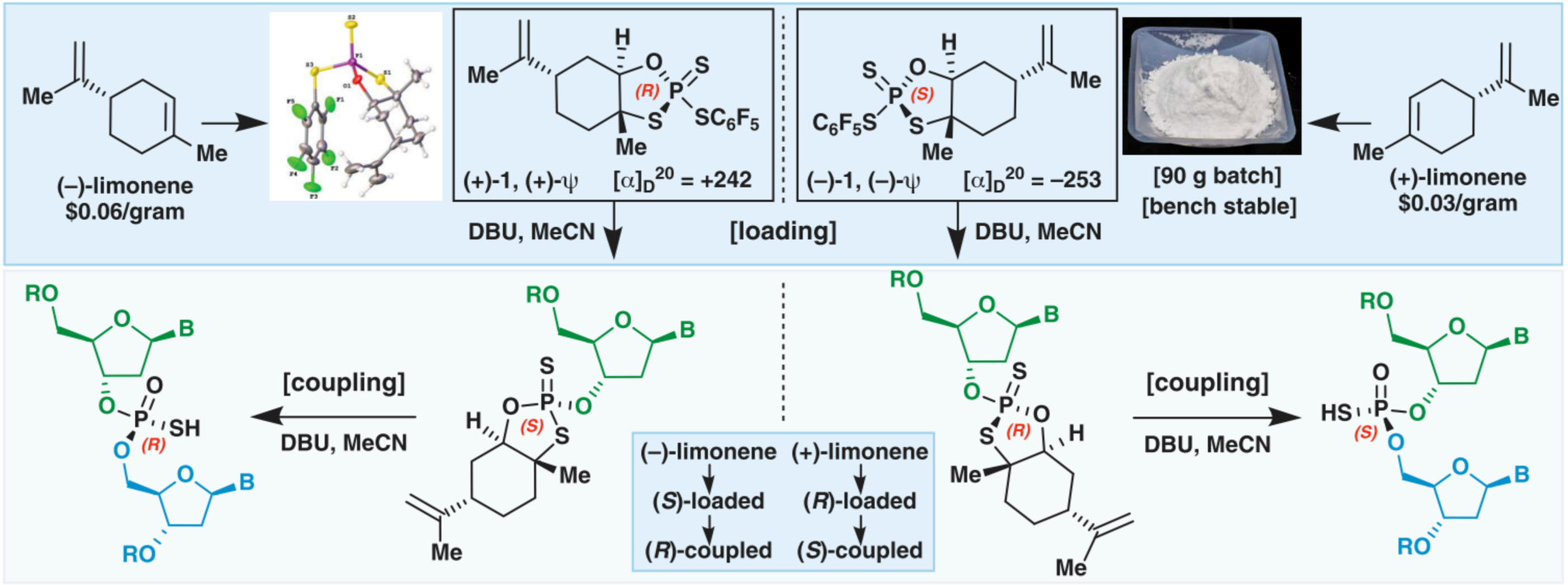
- Summary

Synthesis and Stereochemistry of ψ reagents

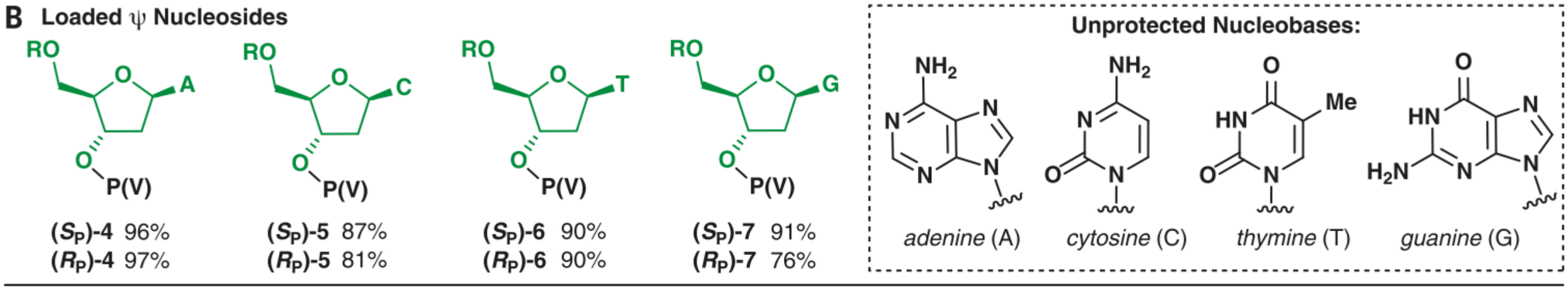
Loading condition: alcohol 1.0 eq., ψ 1.3 eq., DBU 1.3 eq., MeCN, 25 ºC, 30 min

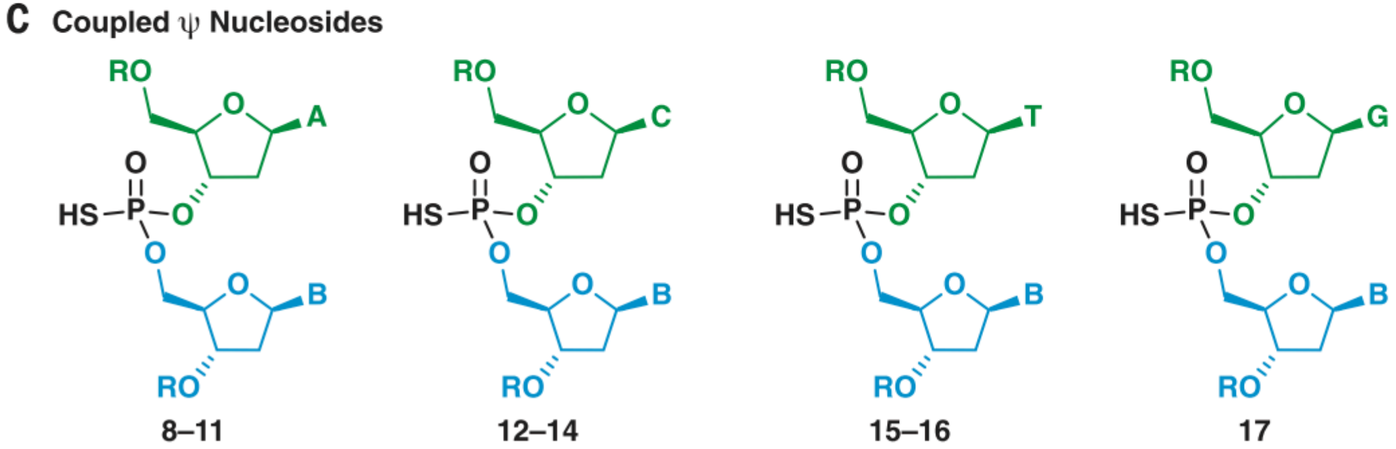


Coupling condition: alcohol 2.0 eq., Nucleoside P(V) 1.0 eq., DBU 3.0 eq., MeCN, 25 ºC, 30 min

Knouse, K. W.; Baran, P.; *et al. Science*, **2018**, *361*, 1234

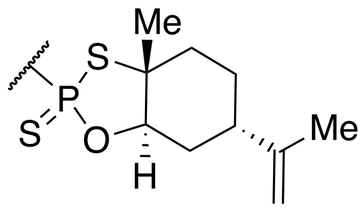
Loading and Coupling of Nucleosides with ψ reagents





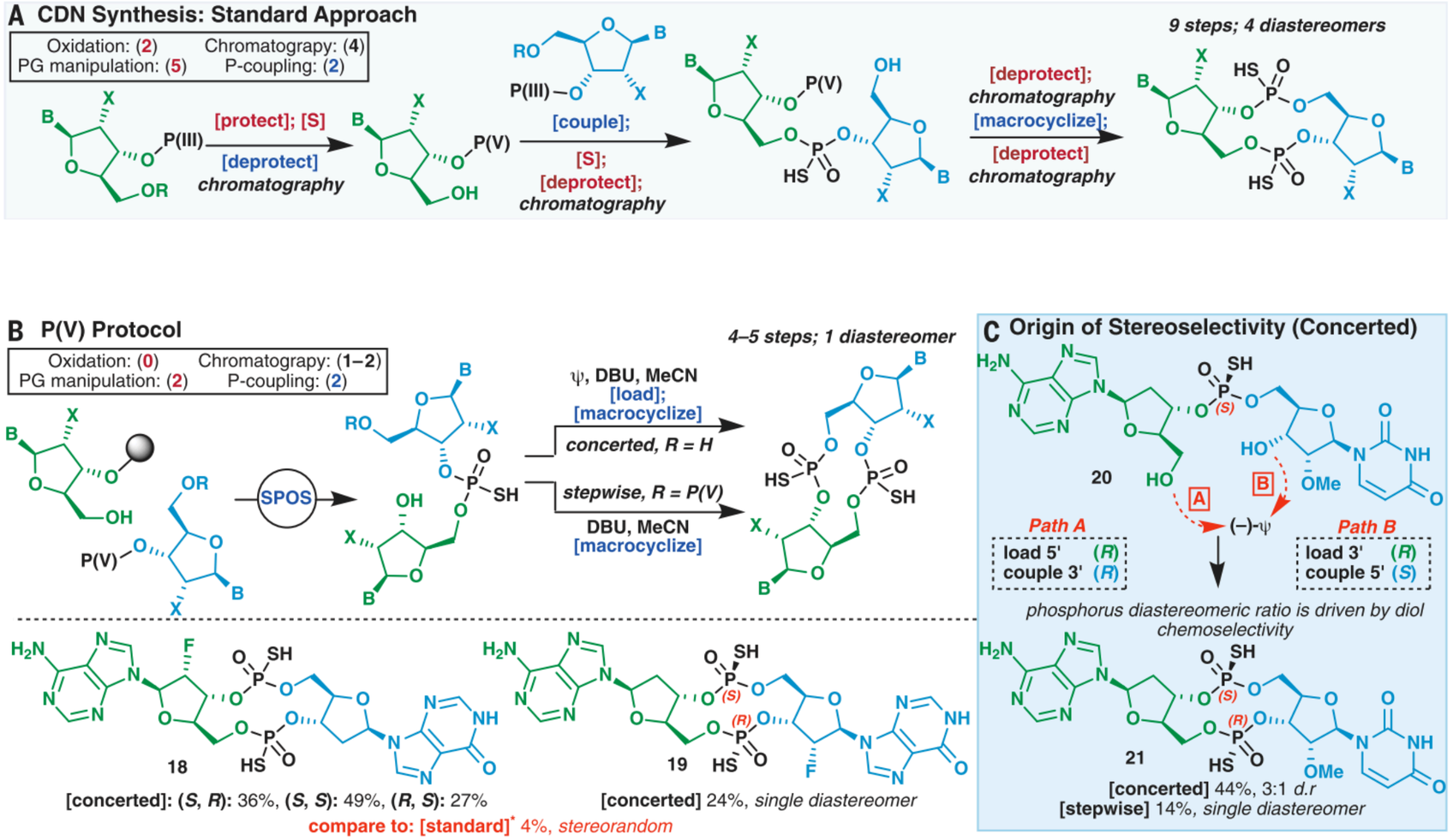
dinucleotide	yield (*d.r.*)
8, dA-dA (R_P)	65% (>99:1)
9, dA-dC (S_P)	91% (>99:1)
10, dA-dT (S_P)	61% (>99:1)
11, dA-dG (S_P)	79% (>99:1)
12, dC-dC (R_P)	73% (>99:1)
13, dC-dT (S_P)	76% (>99:1)
14, dC-dG (R_P)	82% (>99:1)
15, dT-dT (S_P)	72% (>99:1)
16, dT-dG (S_P)	67% (>99:1)
17, dG-dG (R_P)	86% (>99:1)

P(V)



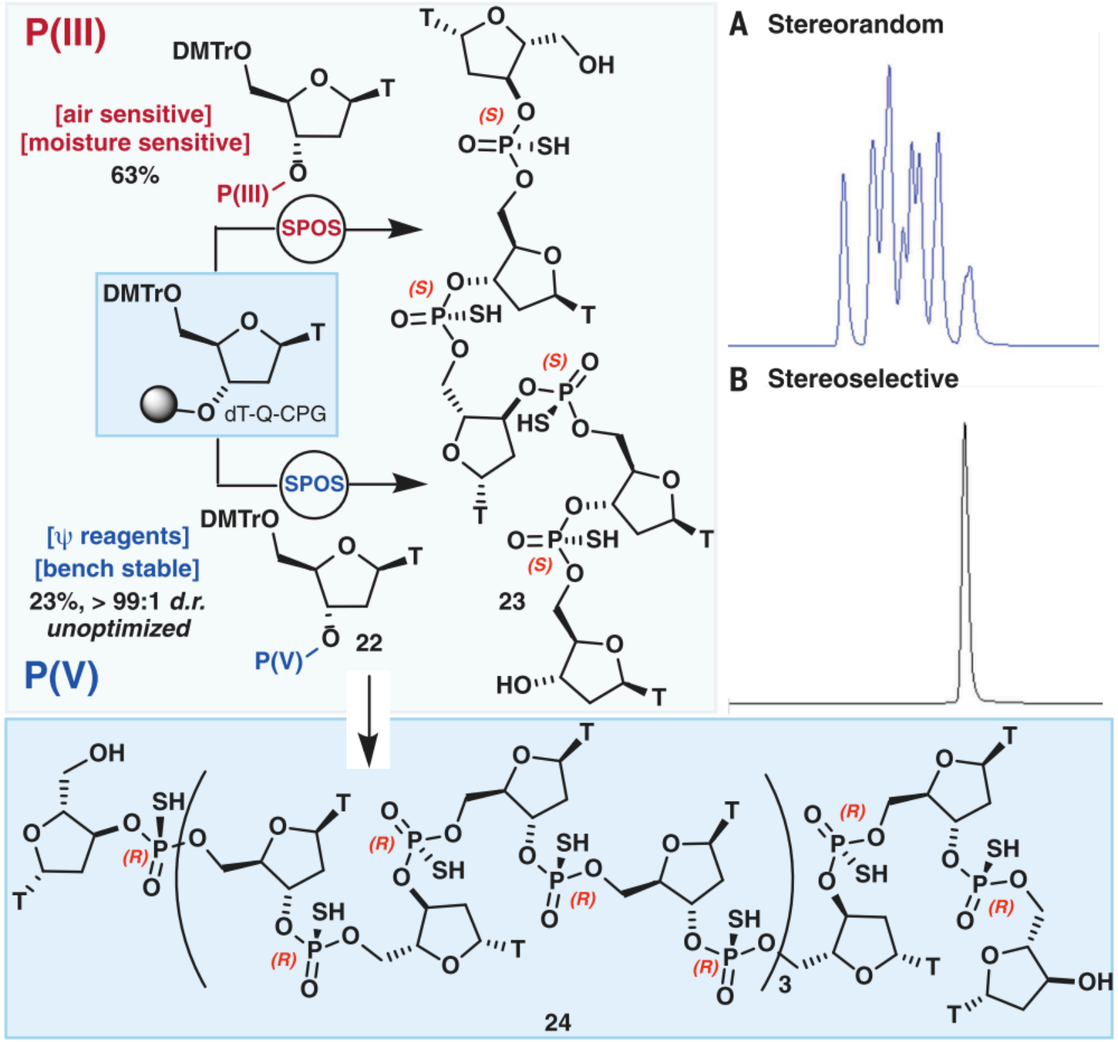
Knouse, K. W.; Baran, P.; *et al. Science*, **2018**, *361*, 1234

CDN Synthesis: Standard approach vs. ψ reagent platform



Knouse, K. W.; Baran, P.; *et al. Science*, **2018**, *361*, 1234

Automated synthesis of PS oligonucleotides



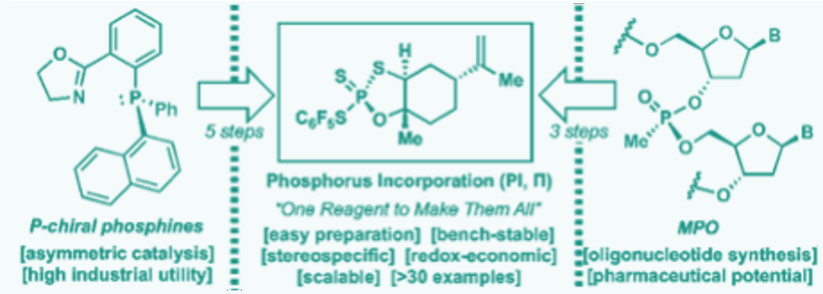
- ψ reagent can be utilized in stereoselective SPOS.

Fig. 4. Automated synthesis of PS oligonucleotides. (**A**) Crude HPLC trace of pentamer **23** (16 diastereoisomers) synthesized under standard P(III) automated conditions. (**B**) Crude HPLC trace of pentamer **23** (1 diastereoisomer) synthesized under unoptimized Ψ automated conditions. DMTr, dimethoxytrityl.

Knouse, K. W.; Baran, P.; *et al. Science*, **2018**, *361*, 1234

Other applications of ψ reagents

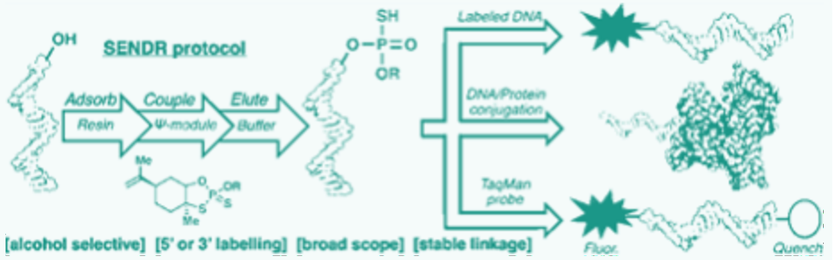
Enantiodivergent formation of C–P bond



Xu, D.; Baran, P.; *et al. J. Am. Chem. Soc.* **2020**, *142*, 5785

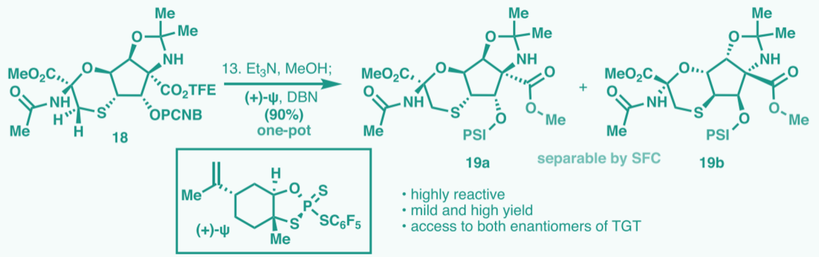
PSI Reagent (1) [phosphorus–sulfur incorporation, ψ]

Native DNA manipulation



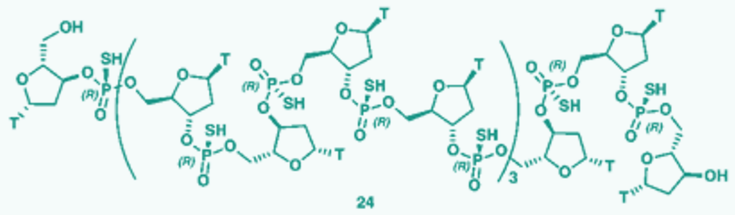
Flood, D. T.; Baran, P.; *et al., ACS Cent. Sci.* **2020**, *6*, 1789

Total Synthesis of Tagetitoxin



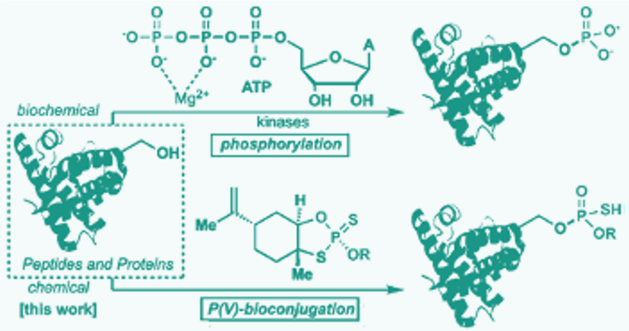
He, C.; Baran, P.; *et al. J. Am. Chem. Soc.* **2020**, *142*, 13683

Oligonucleotide synthesis



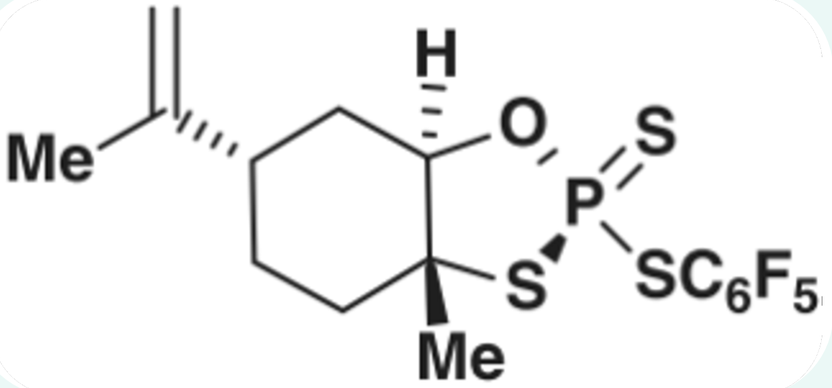
Knouse, K. W.; Baran, P.; *et al. Science*, **2018**, *361*, 1234

Serine selective bioconjugation



Vantourout, J.; Baran, P.; *et al. J. Am. Chem. Soc.* **2020**, *142*, 17236

Short summary



PSI Reagent (1)
[phosphorus–sulfur incorporation, ψ]

- Redox-economic
- Bench-stable reagents
- Simplified protocol
- Solid and solution phase
- Rapid and scale amenable
- Highly broad applications other than ASO synthesis

Knouse, K. W.; Baran, P.; *et al. Science*, **2018**, *361*, 1234

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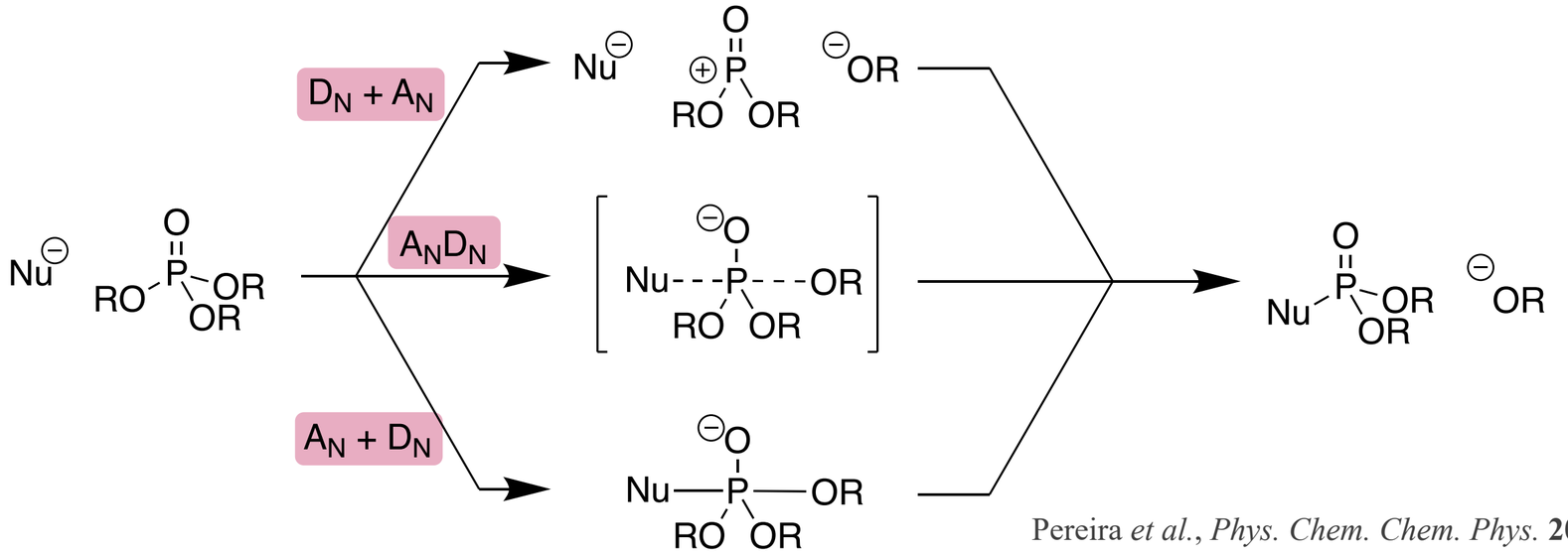
- Stereoselectivity
  - *d.r.* >99:1 in the synthesis of ASO
- Chemoselectivity
  - Serine-selective phosphorylation in the presence of other nucleophiles
- High reactivity under mild condition
  - 30 min at r.t. in loading and coupling reaction

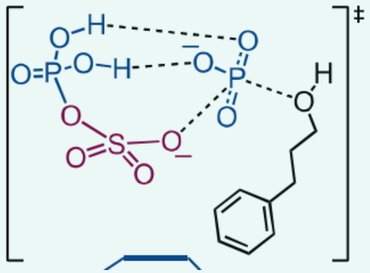
Reaction mechanism of phosphoryl transfer



Pereira *et al.*, *Phys. Chem. Chem. Phys.* **2016**, *18*, 18255

$A_N D_N$

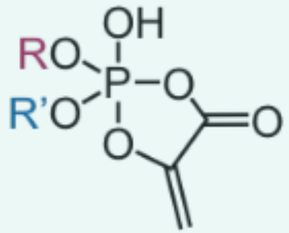
POSOP transition state



Domon, K.; Fujiyoshi, K.; Kanai, M.; *et al.*
ACS Cent. Sci. **2020**, *6*, 283

$A_N + D_N$

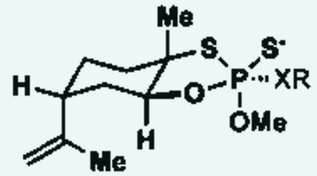
R_2PEP intermediate



Fujiyoshi, K.; Motomu, K.; *et al.*
Synlett **2021**, *32*, 1135

$A_N + D_N$

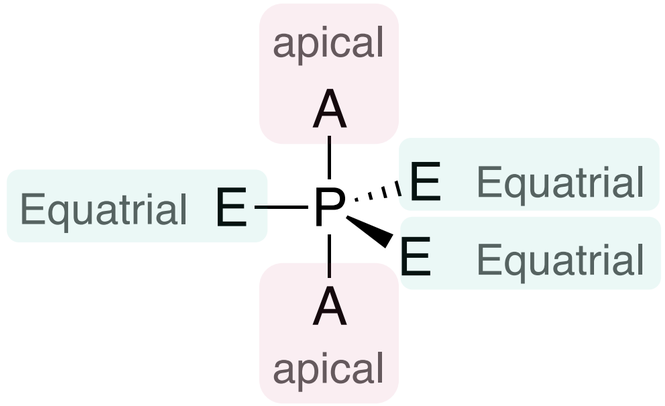
ψ reagent intermediate



INT1
(TBP)

Vantourout, J.; Baran, P.; *et al.*
J. Am. Chem. Soc. **2020**, *142*, 17236

Trigonal-bipyramidal (TBP) phosphorane intermediate

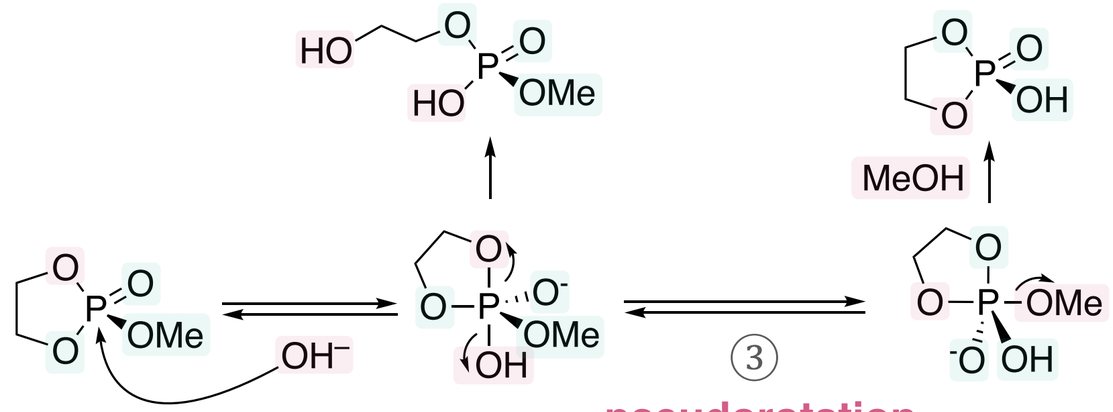


① **Apicophilicity**

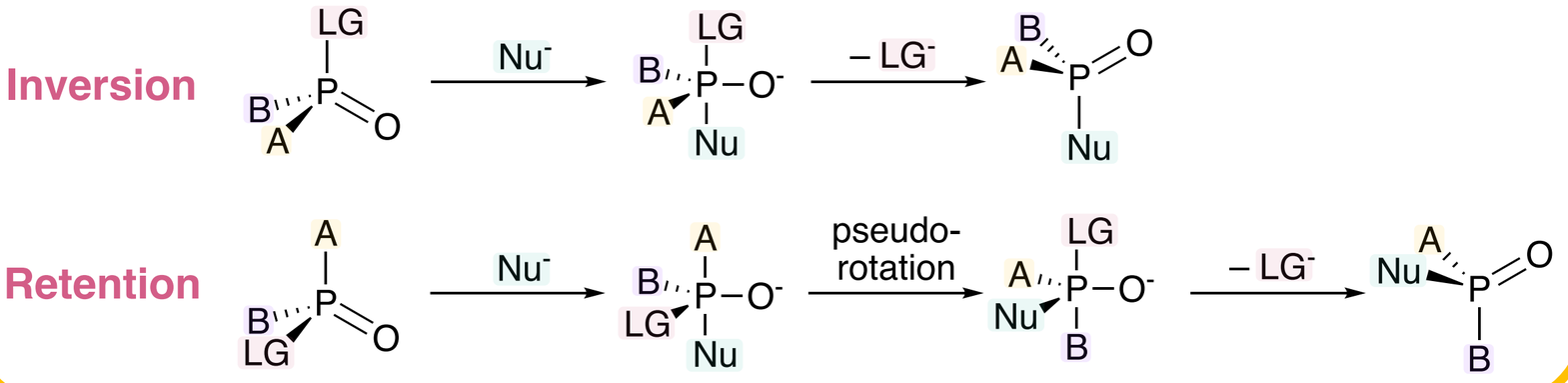
- Electronegative
- Small ligand

F > O > C, N, S

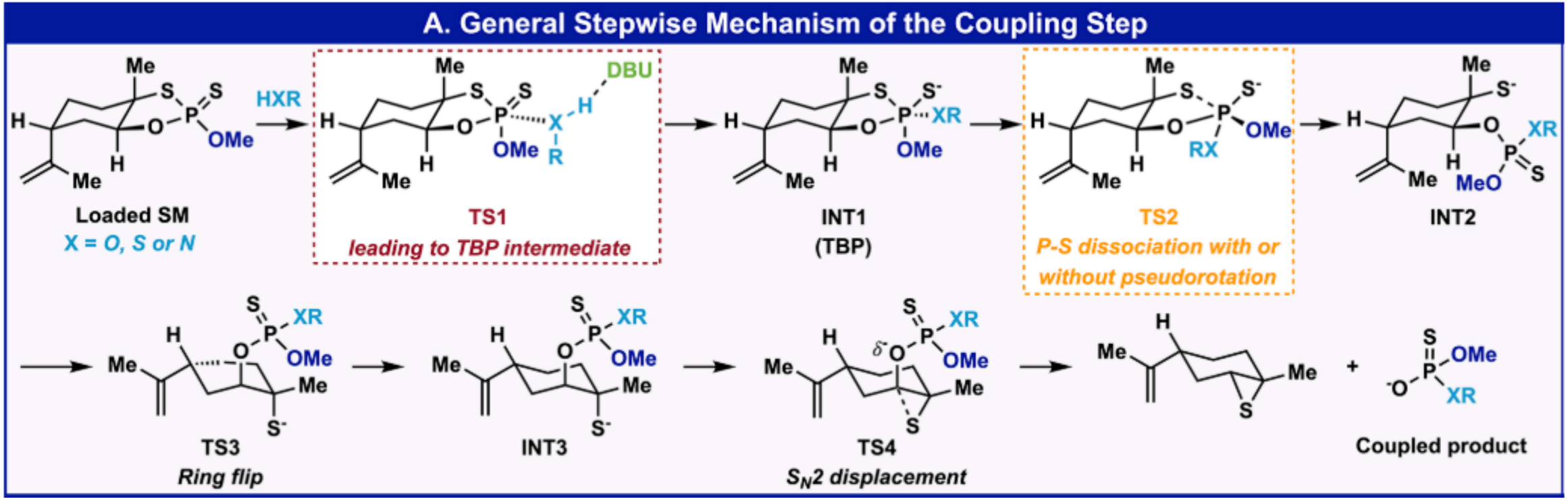
② Addition and elimination occurs from **apical**



④ Configuration at phosphorus



Proposed mechanism

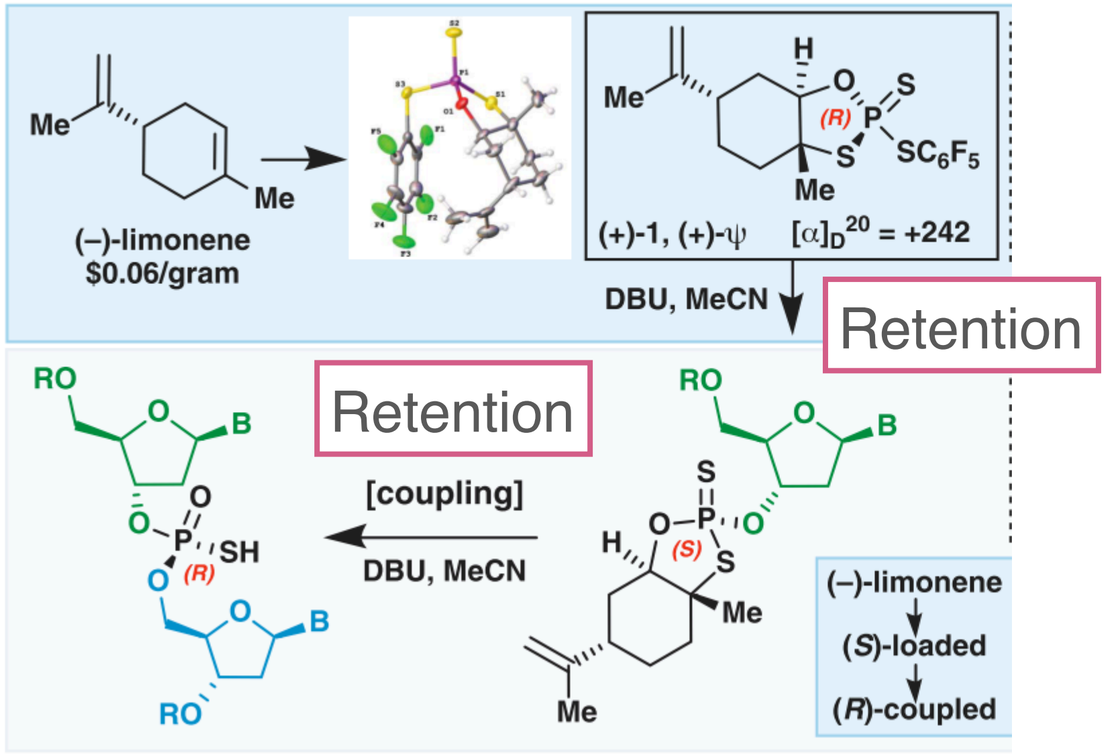


Vantourout, J.; Baran, P.; *et al. J. Am. Chem. Soc.* **2020**, *142*, 17236

The noteworthy points of ψ reagent

- Stereoselectivity
  - *d.r.* >99:1 in the synthesis of ASO
- Chemoselectivity
  - Serine-selective phosphorylation in the presence of other nucleophiles
- High reactivity under mild condition
  - 30 min at r.t. in loading and coupling reaction

Question: The stereochemistry of ψ reagents



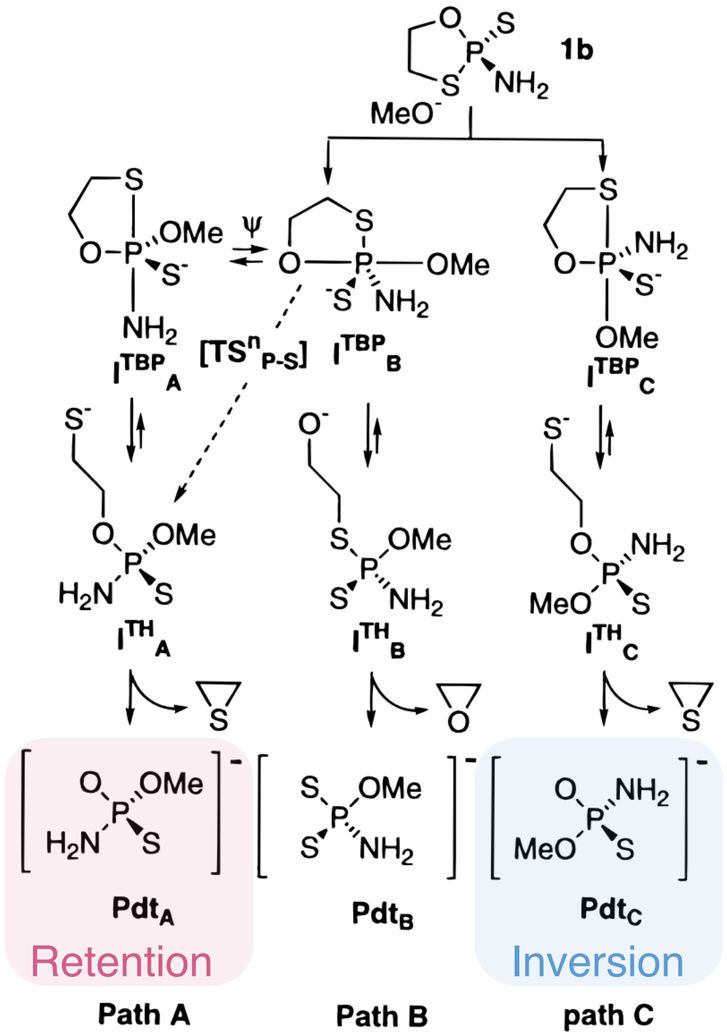
Each loading and coupling step shows a **retention** of configuration at phosphorus.

↓

Why?

Knouse, K. W.; Baran, P.; *et al. Science*, **2018**, *361*, 1234

Possible pathways for methanolysis of the oxathiaphospholane



[a] ψ indicates a pseudorotation process.

The methanolysis of 1b occurrs with a retention of configuration at phosphorus.

↓

- To minimize the ring strain, the five-membered ring occupies one apical and one equatrial position in TBP.
- Anionic S atom derived from P=S is always equatrial.

↓

Three pathways A, B and C are possible.

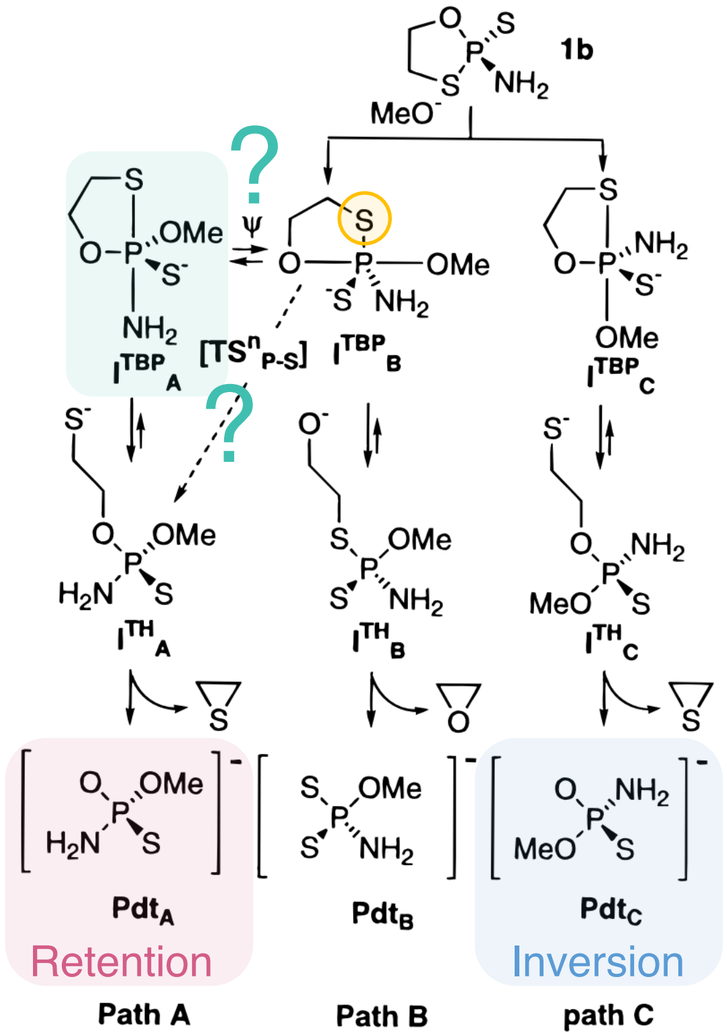
Only path A show a retention of configuration at phosphorus.

↓

However…

Uchimaru, T.; Stec, W.; Taira K. *J. Org. Chem.*, **1997**, *62*, 5793

Possible pathways for methanolysis of the oxathiaphospholane



[a] ψ indicates a pseudorotation process.

Only path A show a retention of configuration at phosphorus.

↓

However…

↓

- I^{TBP}_{A} is unstable compared with I^{TBP}_{B}…
  (S and N are less apicophilic than O)
- The elimination of equatrial ligand
  doesn't occur directly…

↓

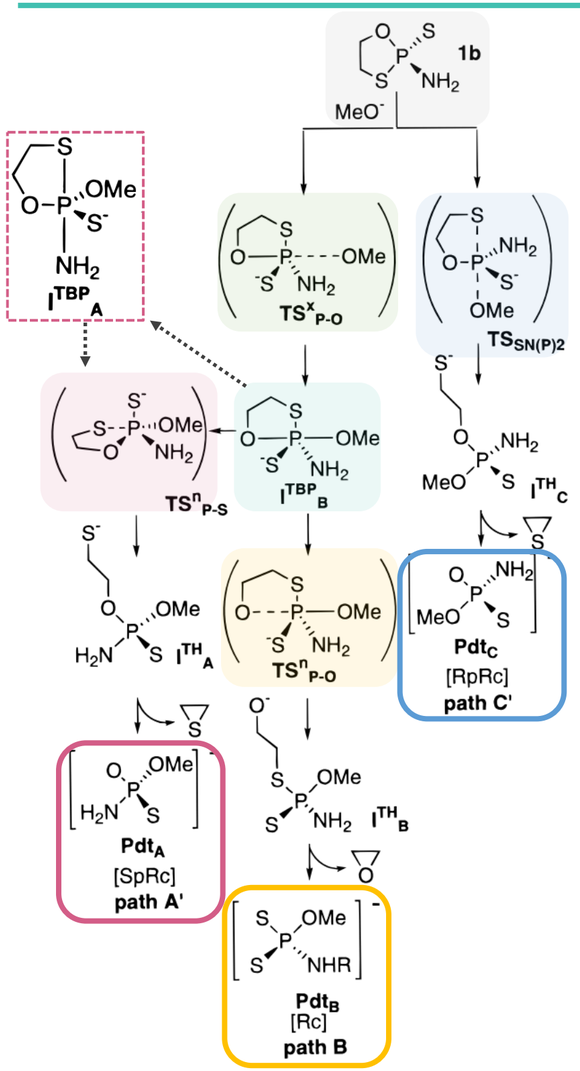
Why does the substitution occur in such a way?

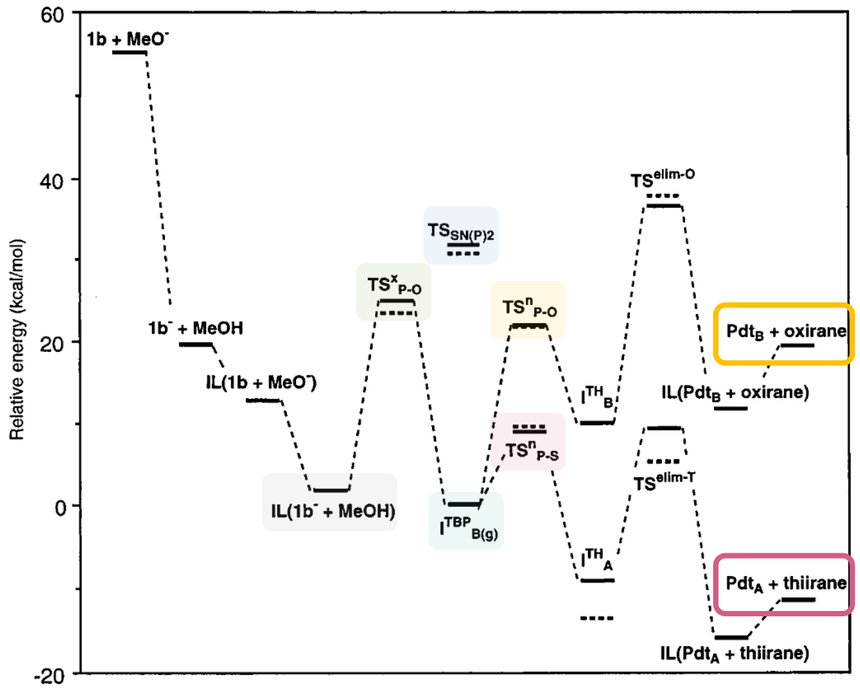
↓

Ab initio caliculation

Uchimaru, T.; Stec, W.; Taira K. *J. Org. Chem.*, **1997**, *62*, 5793

Ab initio calculation



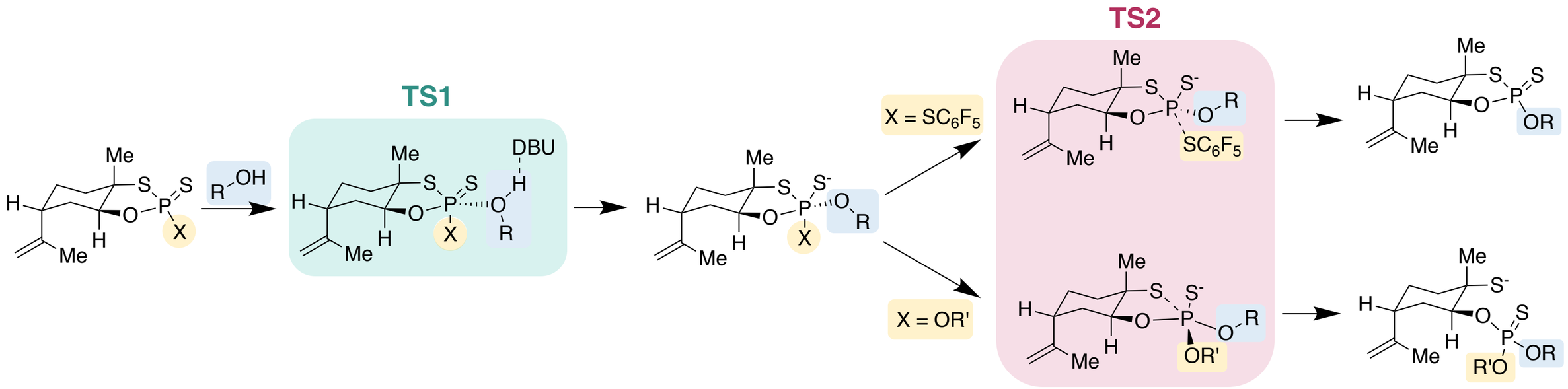


[a] The transition-state structures are shown in parentheses.

1. Nucleophilic attack of methoxide collinear to the endocyclic P-S bond should occur in concert with breaking of the P-S bond. (Path C)
2. Pseudorotation in I^{TBP}_B will occur concomitant with cleavage of the endocyclic P–S bond via the transition state TS^{n}_{P-S} to immediately give I^{TH}_A, without forming I^{TBP}_A. (Path A)
3. Path A will be preffered over either path B or C.

Uchimaru, T.; Stec, W.; Taira K. *J. Org. Chem.*, **1997**, *62*, 5793

Answer: The stereochemistry of ψ reagents



- Nucleophilic attack of alcohol will occur from the opposite side of endocyclic oxygen via TS1.
- Pseudorotation will occur accompanied by the cleavage of the endocyclic P–S bond via TS2.
- These processes lead to the diastereoselective reaction with a retention of configuration at phosphorus.

Uchimaru, T.; Stec, W.; Taira K. *J. Org. Chem.*, **1997**, *62*, 5793

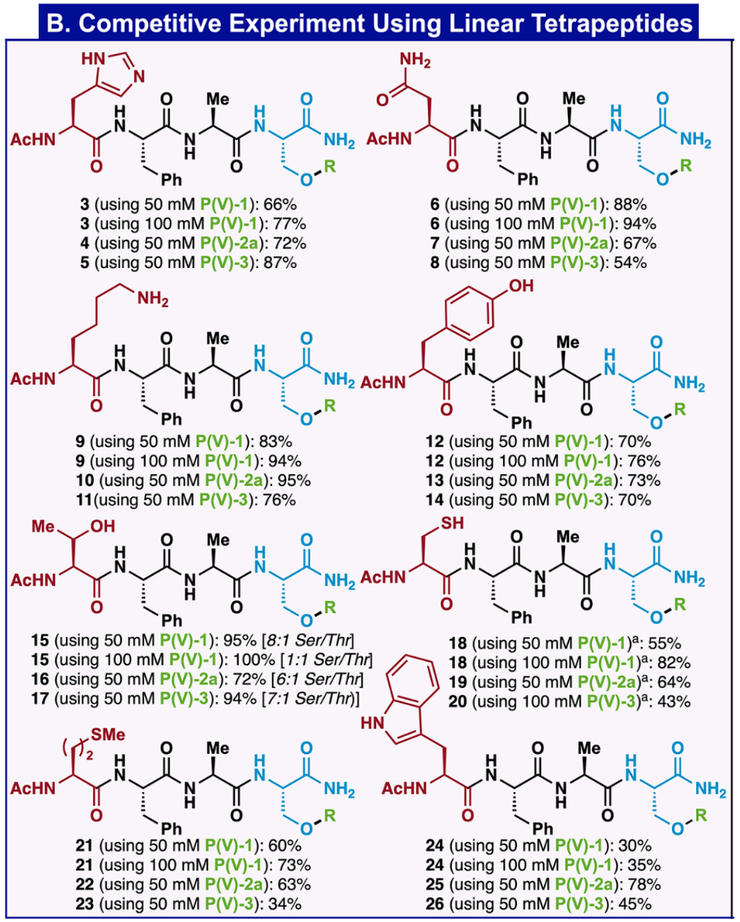
Knouse, K. W.; Baran, P.; *et al. Science*, **2018**, *361*, 1234

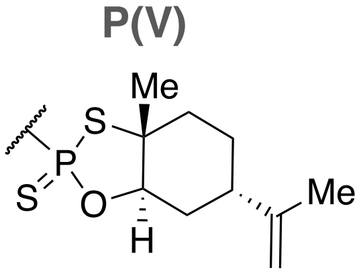
Vantourout, J.; Baran, P.; *et al. J. Am. Chem. Soc.* **2020**, *142*, 17236

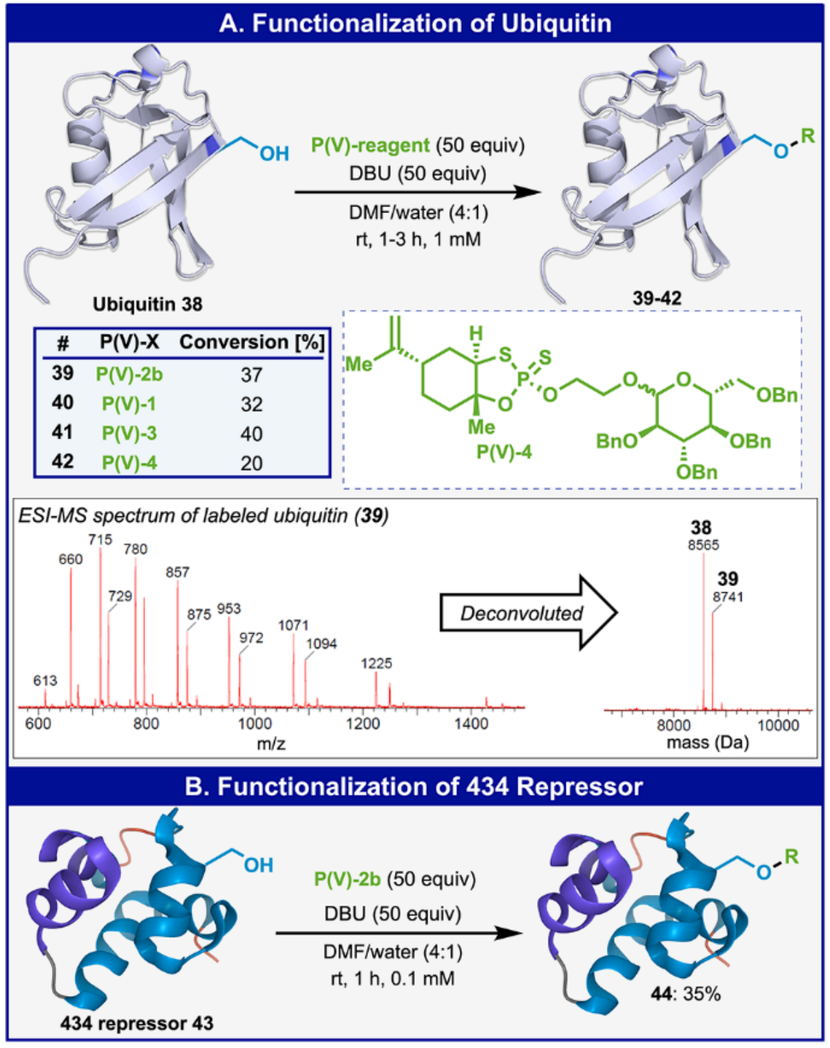
The noteworthy points of ψ reagent

- Stereoselectivity
  - *d.r.* >99:1 in the synthesis of ASO
- Chemoselectivity
  - Serine-selective phosphorylation in the presence of other nucleophiles
- High reactivity under mild condition
  - 30 min at r.t. in loading and coupling reaction

Serine selective bioconjugation

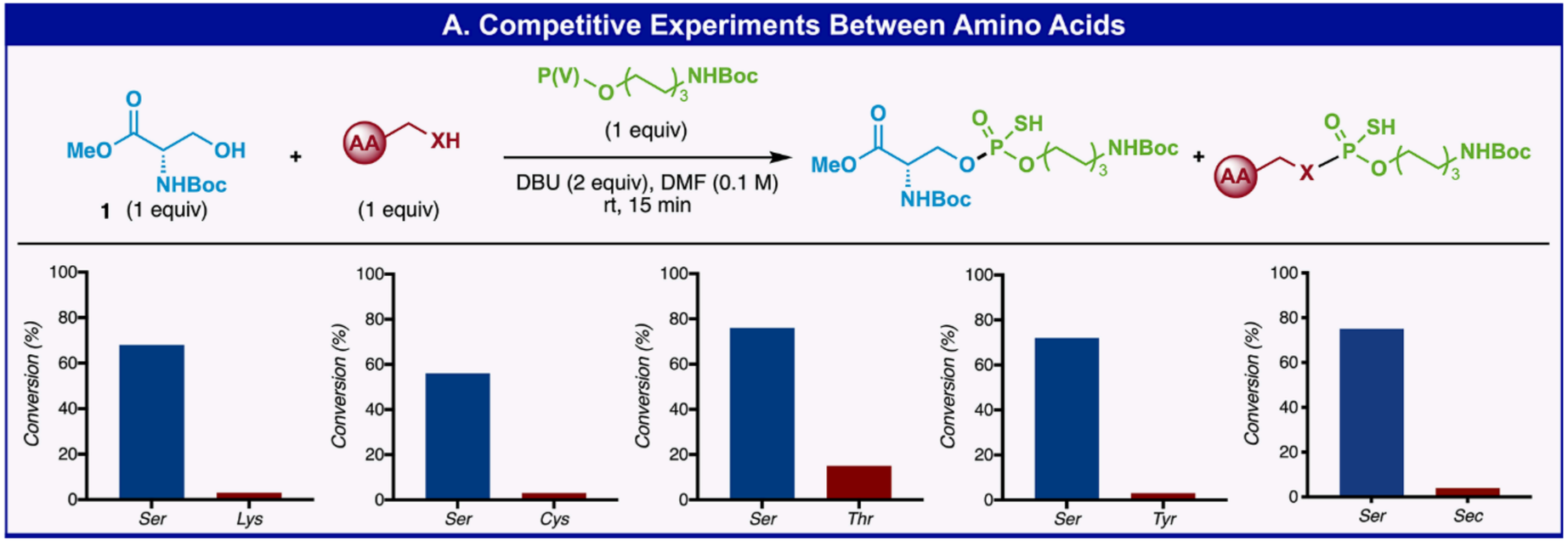






Vantourout, J.; Baran, P.; *et al. J. Am. Chem. Soc.* **2020**, *142*, 17236

Question: The chemoselectivity of ψ reagent



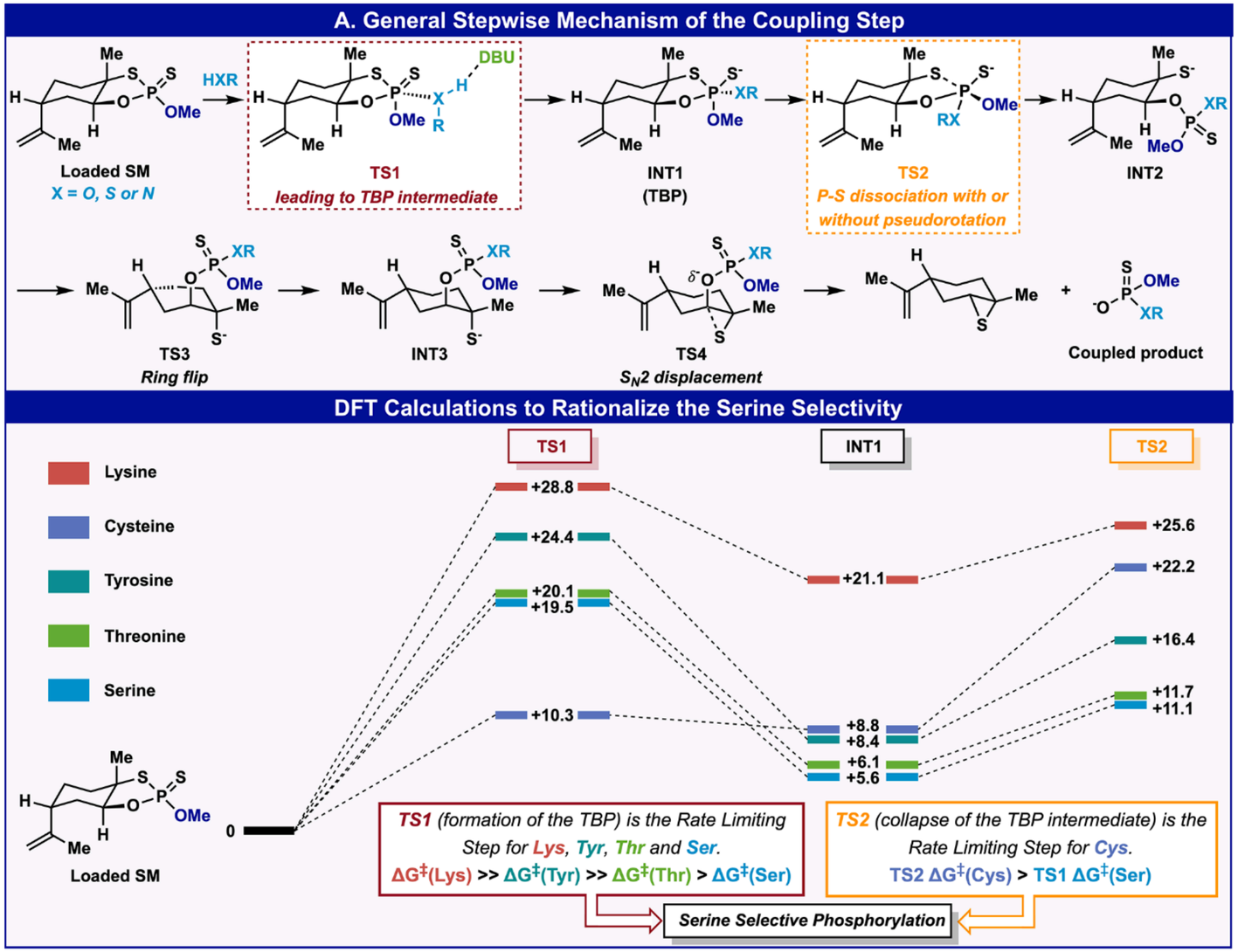
ψ reagent selectively react with serine-OH in the presence of other nucleophiles.

↓

How does ψ reagents distinguish nucleophiles?

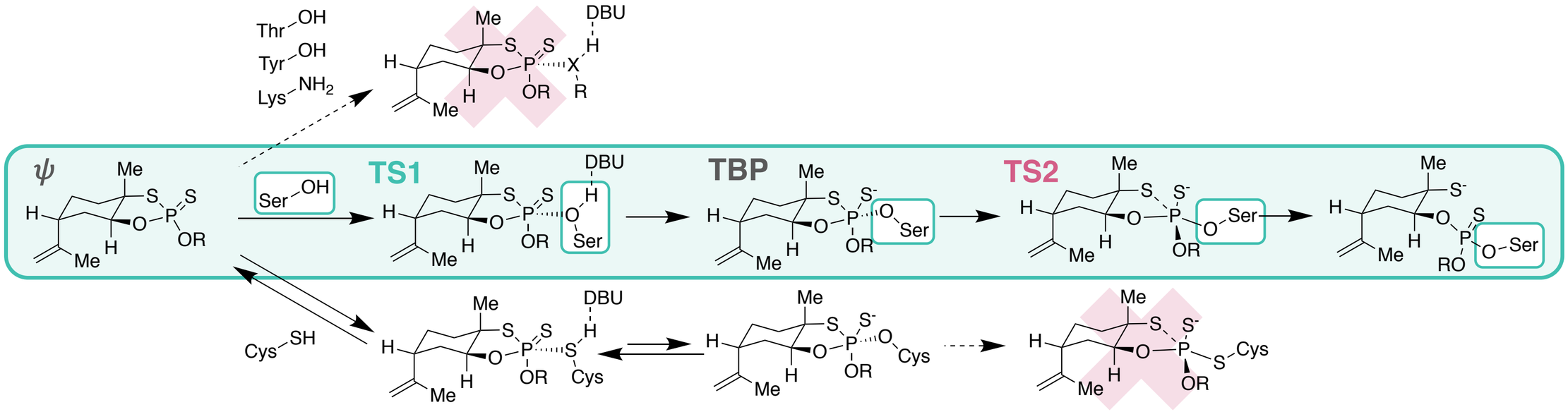
Vantourout, J.; Baran, P.; *et al. J. Am. Chem. Soc.* **2020**, *142*, 17236

DFT calculations to rationalize the serine selectivity



Vantourout, J.; Baran, P.; *et al. J. Am. Chem. Soc.* **2020**, *142*, 17236

Answer: The chemoselectivity of ψ reagent



- Barriers in the formation of TBP intermediate (TS1)
  Cys << Ser < Thr << Tyr (limitted reactivity) << Lys (lower acidity of primary amine)
- For Cys, the collapse of the TBP intermediate is late limitting step.
  TS2 $\Delta G^{\ddagger}$ (Cys) > **TS1** $\Delta G^{\ddagger}$ (Ser)
- These results rationalize the serine selectivity.

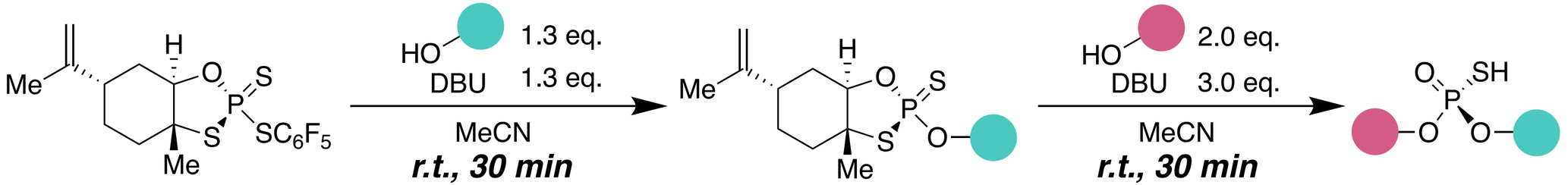
Vantourout, J.; Baran, P.; *et al. J. Am. Chem. Soc.* **2020**, *142*, 17236

The noteworthy points of ψ reagent

- Stereoselectivity
  - *d.r.* >99:1 in the synthesis of ASO
- Chemoselectivity
  - Serine-selective phosphorylation in the presence of other nucleophiles
- High reactivity under mild condition
  - 30 min at r.t. in loading and coupling reaction

Question: The best reactivity of ψ reagent

Bench stable



Knouse, K. W.; Baran, P.; *et al. Science*, **2018**, *361*, 1234

ψ reagent has great stability for air and moisture and is highly reactive under mild condition.

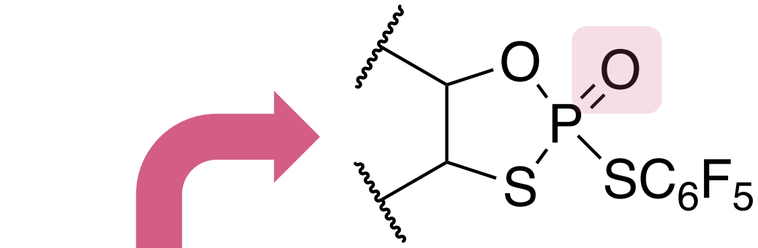
↓

How does ψ reagent realize the balance of stability and reactivity?

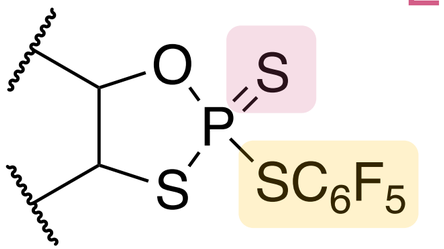
DBU is one of the most effective base for activation of alcohol.

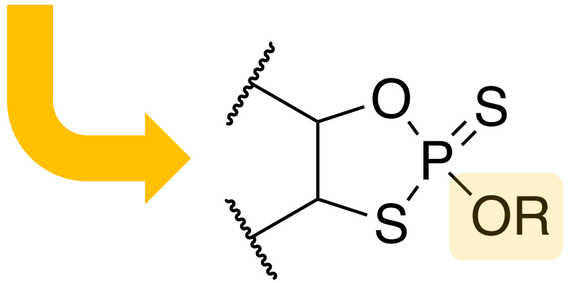
However, is the effect of base only a reason for the high reactivity?

What is the key structure for high reactivity?



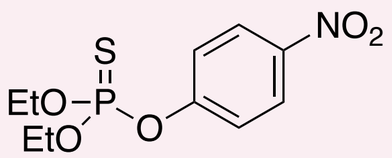
Effect of S?



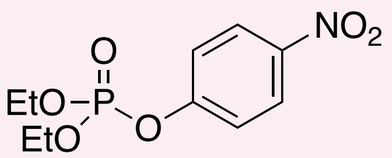


Effect of leaving group?

Parathion



Paraoxon



1.3×10^{-2} < 4.0×10^{-1}

k ($min^{-1}mole^{-1}$) hydrolysis in 50% acetone at 25 ºC

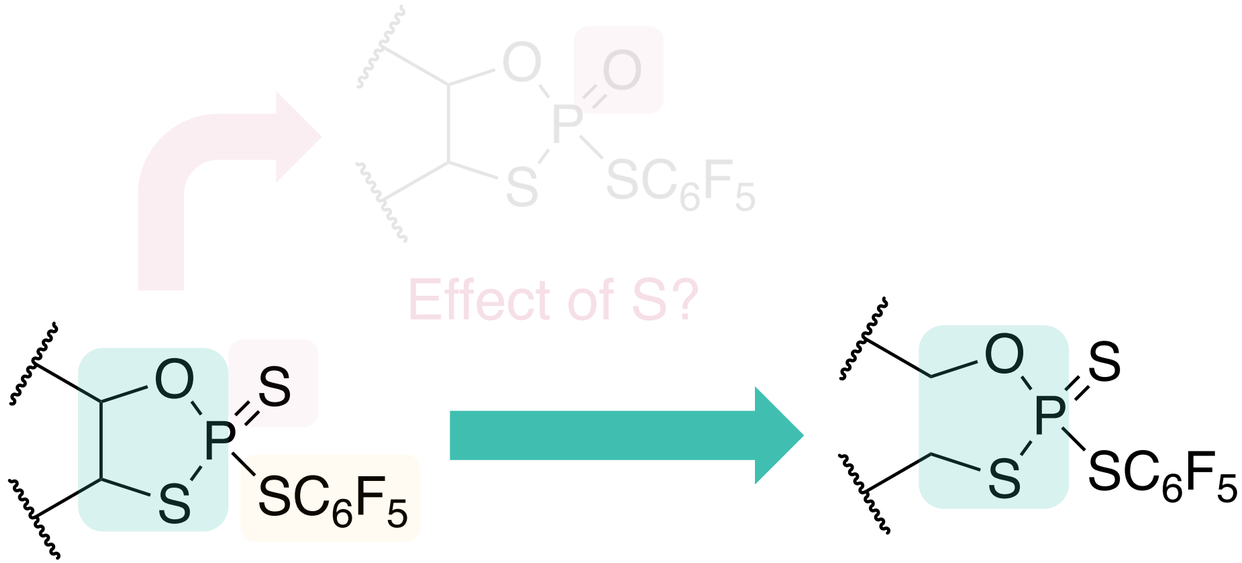
Ketelaar, J.A.A. and Gersmann, H.R. *Recl. Trav. Chim. Pays-Bas*, **1958**,, *77*, 973

The reactions in both of the loading and coupling steps finished in **30 min at r.t.**.

→ The leaving group should not be critical for high reactivity.

Knouse, K. W.; Baran, P.; *et al. Science*, **2018**, *361*, 1234

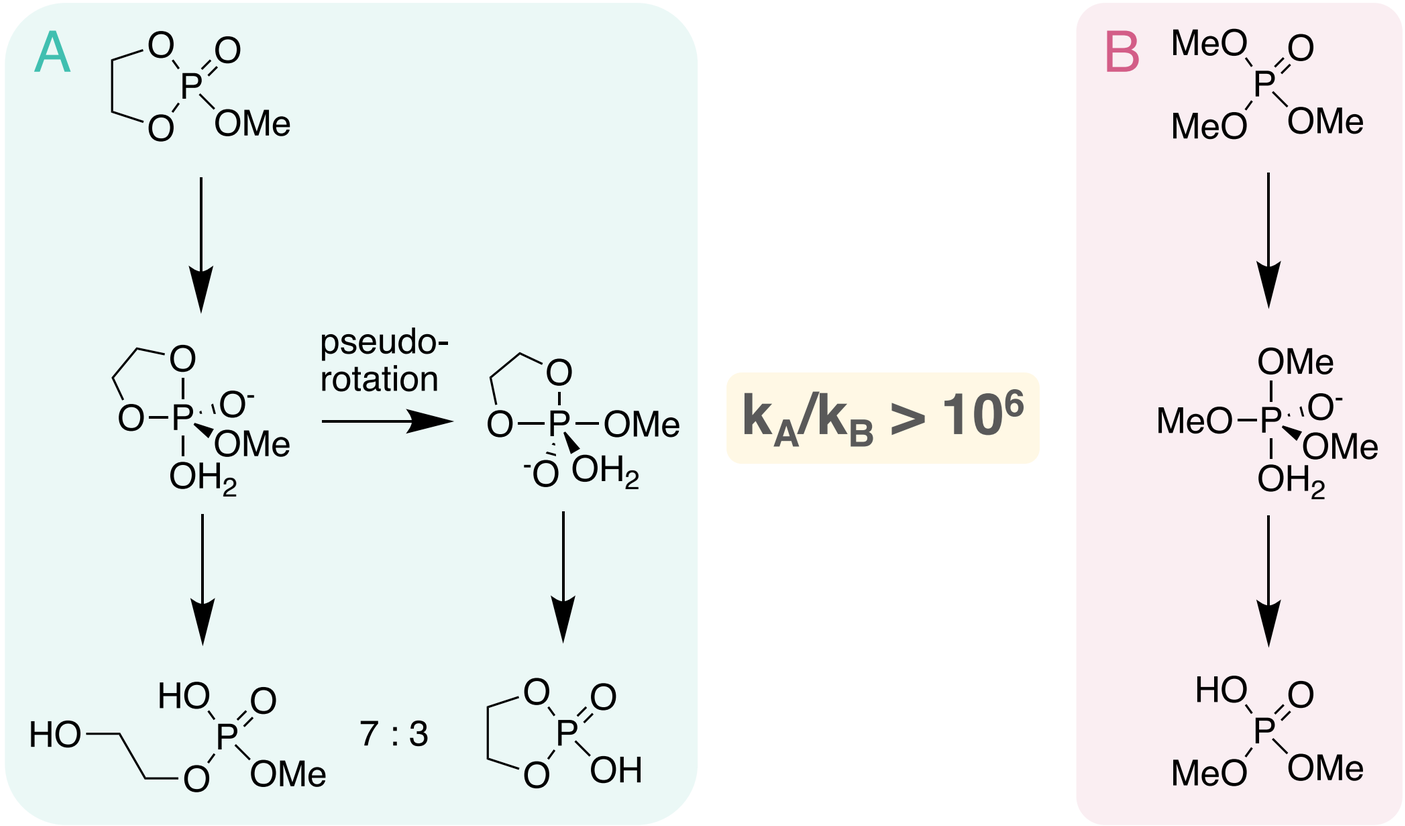
The answer lies in ring moiety



Which is faster for hydrolysis?

Westheimer, F. H. *Acc. Chem. Res.* **1968**, *1*, 70

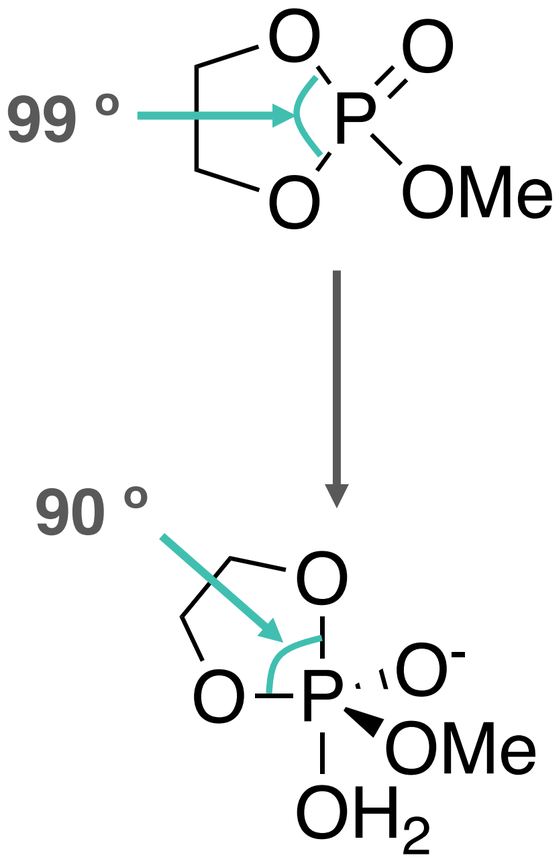
The rate of hydrolysis: Five-membered ring vs. acyclic phosphate



The rate of hydrolysis of methyl ethylene phosphate A (k_A) exceeds that of trimethyl phosphate B (k_B) by ***10^6***.

Westheimer, F. H. *Acc. Chem. Res.* **1968**, *1*, 70

TBP intermediate of five-membered ring phosphate in hydrolysis



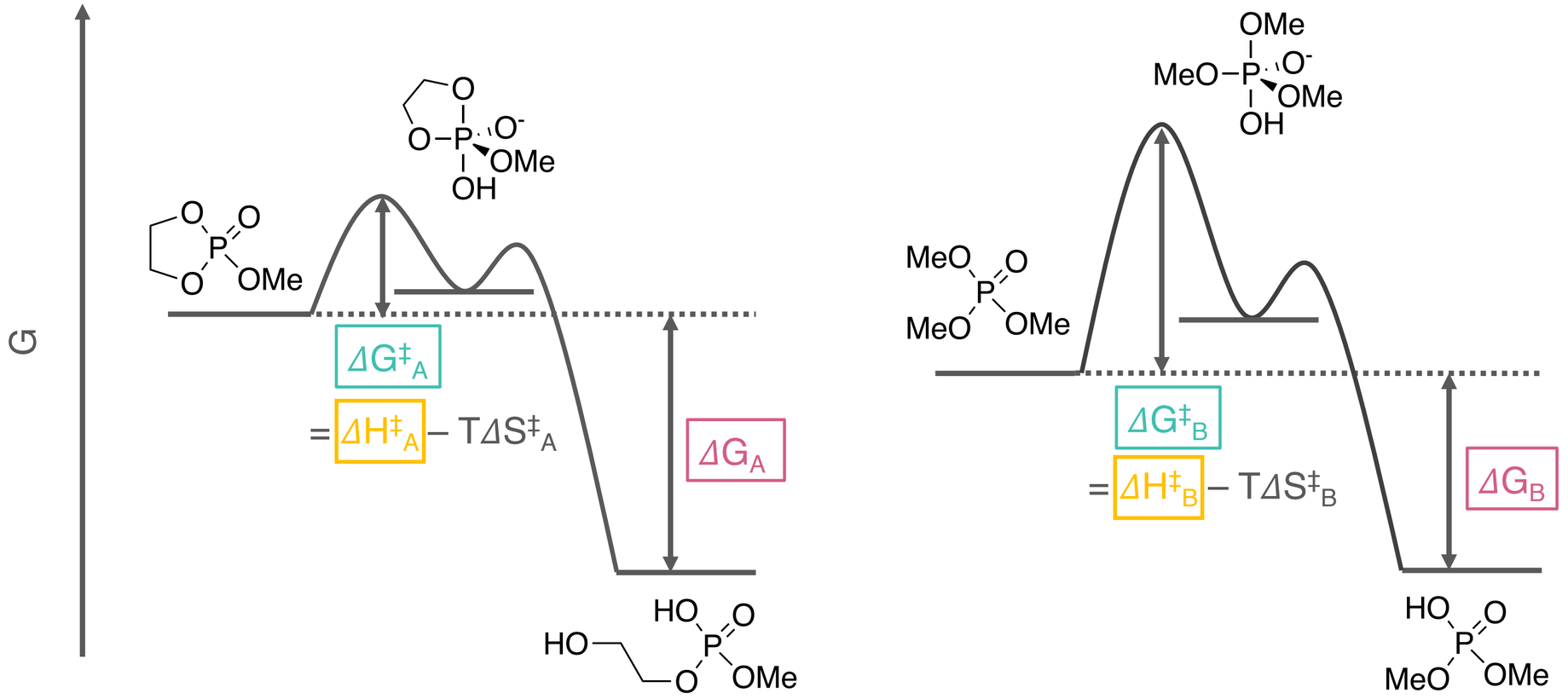
The ring angle at phosphorus (O-P-O) is **99 °** determined by X-ray crystallography.
The five-membered ring is highly strained.

↓

The formation of the TBP intermediate with a **90 °** angle at phosphorus (ring in the equatrial–apical conformation) largely decrease the strain energy.

Westheimer, F. H. *Acc. Chem. Res.* **1968**, *1*, 70

Driving force: ring strain



$\Delta G_A - \Delta G_B$
= 5~6 kcal/mol

$\Delta G^{\ddagger}_A - \Delta G^{\ddagger}_B$
= – 8.5 kcal/mol

$\Delta H^{\ddagger}_A - \Delta H^{\ddagger}_B$
= – 7.6 kcal/mol

The difference of $\Delta H^{\ddagger}$ is comparable to that of $\Delta G^{\ddagger}$.

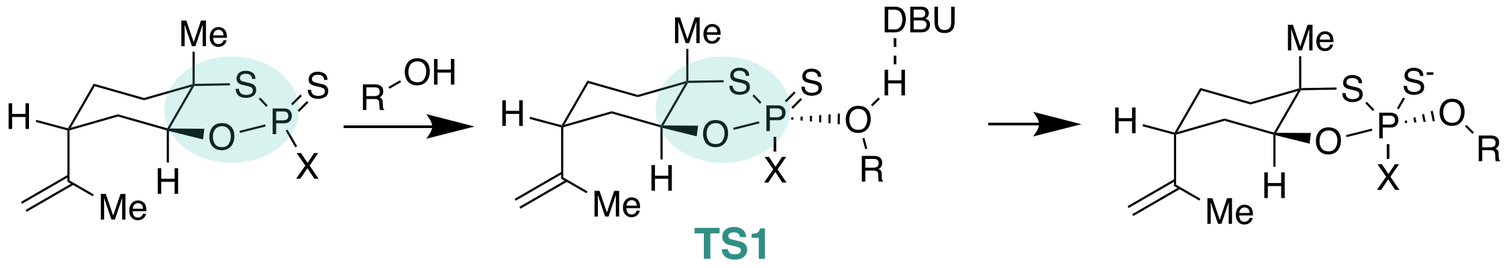
The release of ring strain accounts for the rapid hydrolysis of five-membered ring phosphate A compared with acyclic one B.

Westheimer, F. H. *Acc. Chem. Res.* **1968**, *1*, 70

Chang, N.; Lim, C. *J. Am. Chem. Soc.* **1998**, *120*, 2156

Answer: The best reactivity of ψ reagent

Ring strain → Decrease the enthalpy of activation in rate limitting step → **Ring strain free**

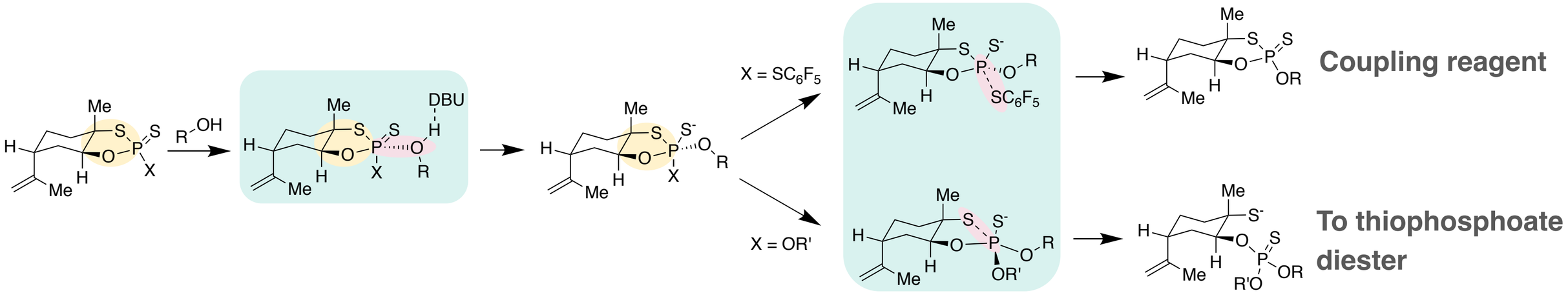


The driving force of ψ reagent should be the ring strain.

It should decrease the activation energy compared with acyclic phosphate.

This is why ψ reagent shows the high reactivity.

Summary



Stereoselectivity

1)The direction of nucleophilic addition

2)The cleavage of P–S bond accompanied by pseudorotation

Chemoselectivity

1)$\Delta G^{\ddagger}$ in TS1

Cys << Ser < Thr << Tyr << Lys

2)TS2 $\Delta G^{\ddagger}$ (Cys) > TS1 $\Delta G^{\ddagger}$ (Ser)

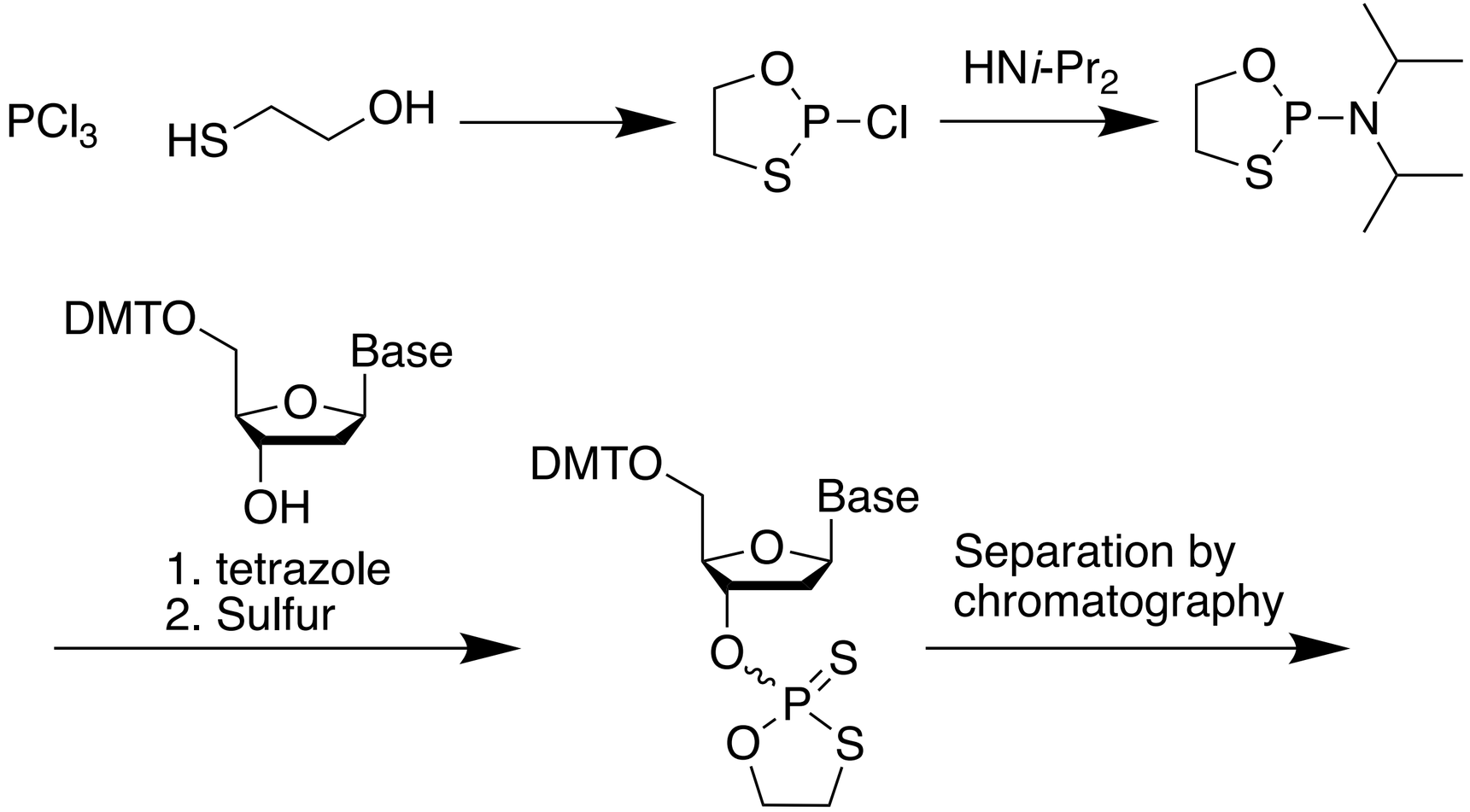
High reactivity

1)Strained five membered ring

2)Lower the entalpy of the activation in TS1

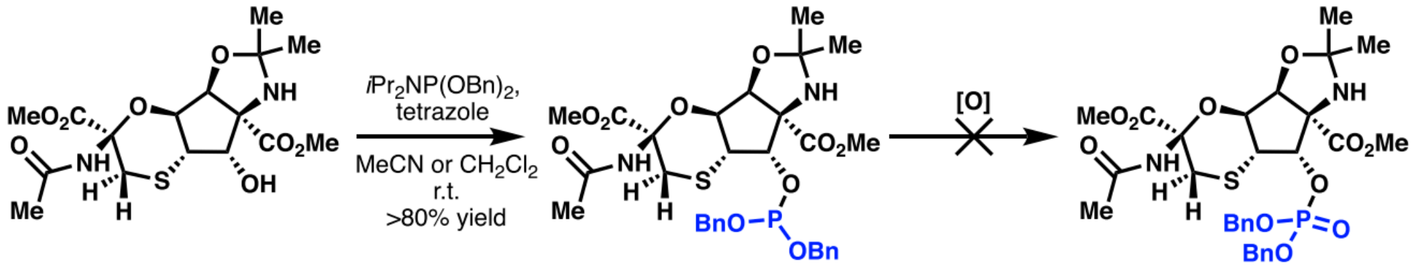
Appendix

Appendix: Synthesis of 5’-O-DMT-nucleoside 3’-O-(2-thio-1,3,2-oxathiaphospholane)



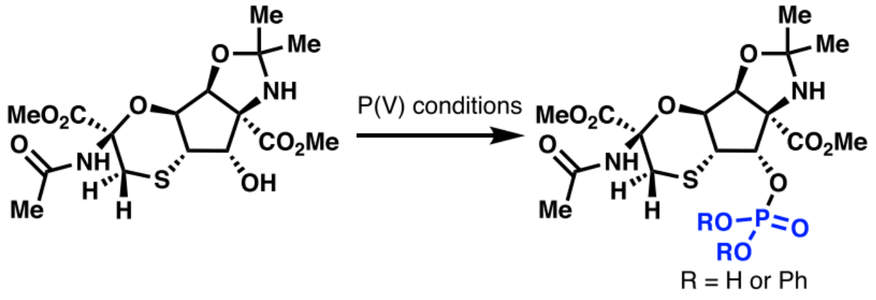
Appendix: Phosphorylation of existing method

a) P(III) to P(V) strategy: *Oxidation was difficult to control. S tends to get over oxidized.*



• H_2O_2, 0 °C, 10 min	sulfur got fully over oxidized to sulfoxide
• *m*CPBA, 0 °C 10 min	sulfur got fully over oxidized to sulfoxide
• I_2, pyridine, 0 °C, 10 min	messy

b) Conventional P(V) strategy: *conditions were either too harsh or invalid.*



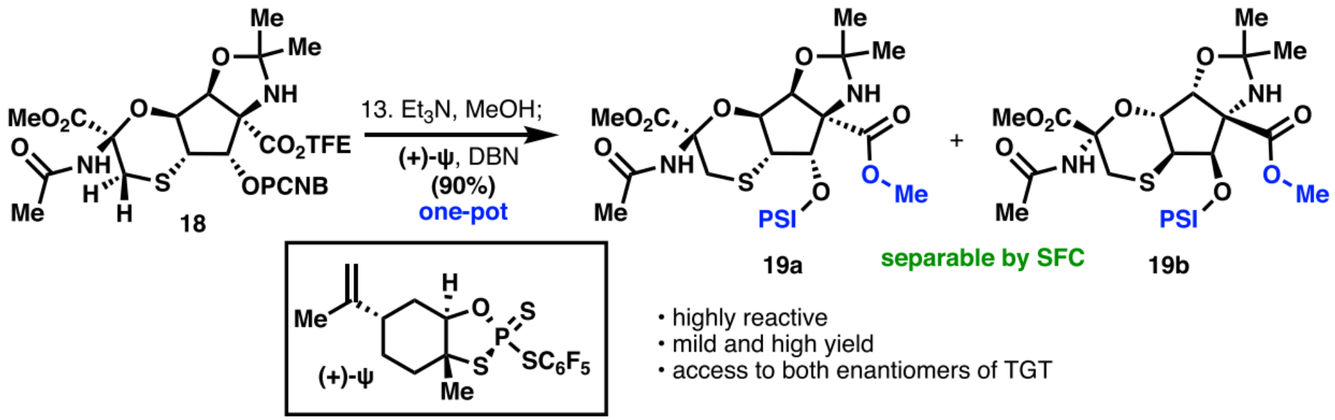
• $POCl_3$, Et_3N, THF, 0 °C; H_2O	decomposition
• $Bu_4N^+H_2PO_4^-$, Cl_3CCN, CH_3CN, rt	decomposition
• $(PhO)_2POCl$, Pyr., CH_2Cl_2, rt	no reaction
• $(PhO)_2POCl$, *t*BuOLi, THF, -78 °C - rt	full conversion of SM, trace of product
• $(PhO)_2POCl$, Et_3N, $TiCl_4$ (cat.), THF, rt	no reaction

The introduction of phosphoryl group to a secondary alocohol by existing methods was quite difficult.

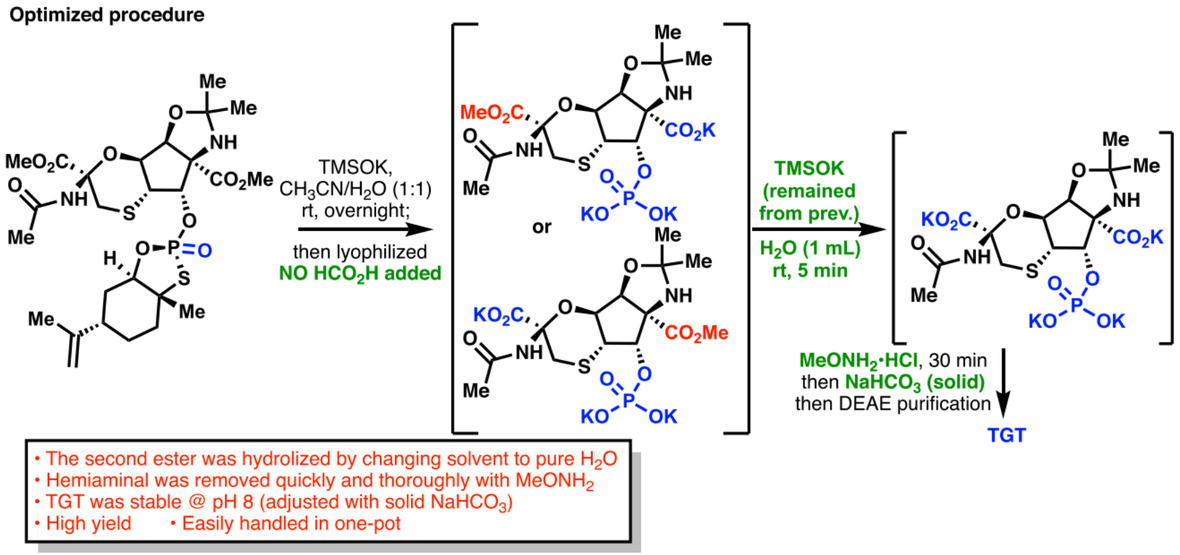
- Phosphoramidite
  - Sulfide was oxidized…
- Other reagents
  - Too harsh or invalid…

Appendix: Phosphorylation by ψ reagent

c) Final phosphorylation with (+)-ψ:



Scheme S20. Optimization of phosphorylation



Ψ reagent provided thiophosphate triester in high yield (90%).

SeO_2 oxidation and hydrolysis of phosphate thioester gave a phosphomonoester final product Tagetitoxin.

Appendix: The rate of hydrolysis of some phosphorus acid esters

Table

Results of hydrolysi measurements

No.	Compound	Solvent	log. freq. factor (min^{-1})	Activation Energy cal/mole	Activation Entropy (Entr. Units)	Free Energy of Activation cal/mole	k(25° C) $min^{-1}mole^{-1}$	k(37.5° C) $min^{-1}mole^{-1}$	Inhibition Cholinesterase* I_{50}	I_{50} Ali-esterase*) / I_{50} Cholinesterase
1	$(C_2H_5O)_3PO$	H_2O	7.94	15000	−32.2	24100	7.9×10^{-4}	2.24×10^{-3}	$> 10^{-1}$	—
2	$(C_2H_5O)_2(C_6H_5O)PO$	50% ethanol	8.76	15000	−28.5	23900	5.3×10^{-3}	1.48×10^{-2}	10^{-3}	3
3	$(C_2H_5O)_2(p\text{-}O_2NC_6H_4O)PO$	50% acetone	8.64	12400	−29.0	20500	4.0×10^{-1}	9.5×10^{-1}	10^{-8}	20
4	$(C_2H_5O)_2(m\text{-}[N^+(CH_3)_3]C_6H_4O)PO$	50% ethanol	11.90	15800	−14.2	19500	1.86	5.5	8×10^{-9}	5×10^{-8}
5	$(C_2H_5O)(p\text{-}O_2NC_6H_4O)_2PO$	50% acetone	11.96	14300	−13.9	17900	3.1×10	8.2×10	2×10^{-8}	10
6	$(CH_3O)(p\text{-}O_2NC_6H_4O)_2PO$	50% acetone	11.25	13400	−17.2	17900	3.4×10	7.8×10	4×10^{-8}	5
7	$(p\text{-}O_2NC_6H_4O)_2P(O)OH$	20% ethanol	10.01	18300	−22.8	24500	3.5×10^{-4}	1.2×10^{-3}		
8	$(p\text{-}O_2NC_6H_4O)_2C_2H_5PO$	20% ethanol	8.97	8700	−27.5	16400	3.7×10^{2}	6.8×10^{2}	5×10^{-7}	0.14
9	$(C_6H_5O)_3PO$	75% ethanol	13.57	16100	− 6.6	17500	5.4×10	1.6×10^{-2}	$> 10^{-2}$	< 0.15
10	$(p\text{-}O_2NC_6H_4O)_3PO$	50% acetone	6.34	4100	−39.6	15400	2.0×10^{3}		4×10^{-5}	1
11	$p\text{-}ClC_6H_4OP(O)(NHC_2H_5)_2$	50% ethanol	10.85	16700	−19.0	21800	3.6×10^{-2}	1.1×10^{-1}	10^{-2}	3×10^{-3}
12	$(p\text{-}ClC_6H_4O)_2P(O)NHCH_3$	50% ethanol	12.01	15200	−13.7	18700	6.8	1.9×10	1.5×10^{-3}	1.4×10^{-3}
13	$(CH_3O)_2(p\text{-}O_2NC_6H_4O)PS$	50% acetone	11.26	16700	−17.1	21200	8.5×10^{-2}	3.4×10^{-1}	5×10^{-4}	0.8
	"	H_2O	10.76	15450	−19.6	20700				
14	$(C_2H_5O)_2(p\text{-}O_2NC_6H_4O)PS$	50% acetone	11.96	19200	−13.9	22700	1.3×10^{-2}	5.5×10^{-2}	(?) 2.5×10^{-5}	1.5
	"	H_2O	10.95	16600	−18.5	21600				
15	$(C_2H_5O)(p\text{-}O_2NC_6H_4O)C_6H_5PS$	20% ethanol	13.79	17500	− 5.6	18600	9.1	2.7×10		
16	$(p\text{-}O_2NC_6H_4O)_2(C_2H_5O)PS$	50% acetone	8.73	12100	−28.6	20100	9.7×10^{-1}	1.8	4×10^{-3}	0.25
17	$(p\text{-}O_2NC_6H_4O)_2(CH_3O)PS$	50% acetone	10.33	13900	−21.3	19700	1.35		1×10^{-3}	
18	$(p\text{-}O_2NC_6H_4O)_3PS$	50% acetone	5.21	5700	−44.7	18500	1.25×10		2×10^{-4}	0.5
19	$(CH_3O)_2([(CO_2C_2H_5)_2CH_2CH]S)PS$	25% acetone	20.45	24500	−24.75	16500	2.6×10^{2}	1.4×10^{3}	(?) 5×10^{-6}	15

*) These values were taken from the work of *Mendel* and *Myers* [9].

Appendix: Ab initio calculations for MEP vs. TMP or EPP

Table 7. Experimental Activation Parameters (kcal/mol) for the Alkaline Hydrolyses of Acyclic TMP, Five-Membered Ring MEP, and 6-Membered Ring EPP at 298.15 K

molecule	$\Delta H^{\ddagger}$	$T\Delta S^{\ddagger}$	$\Delta G^{\ddagger}$
TMP[a]	15.60 ± 0.2	−6.86 ± 0.3	22.46 ± 0.3
MEP[a]	7.80 ± 0.2	−6.86 ± 0.3	14.66 ± 0.3
EPP[b]	13.21	−8.74	21.94
TMP−MEP	7.8 ± 0.4	0.00 ± 0.6	7.80 ± 0.6
EPP−MEP	5.4 ± 0.4	−1.88 ± 0.6	7.29 ± 0.6

[a] Values are taken from ref 12. [b] Values are taken from ref 16. No errors were reported for the activation parameters obtained.

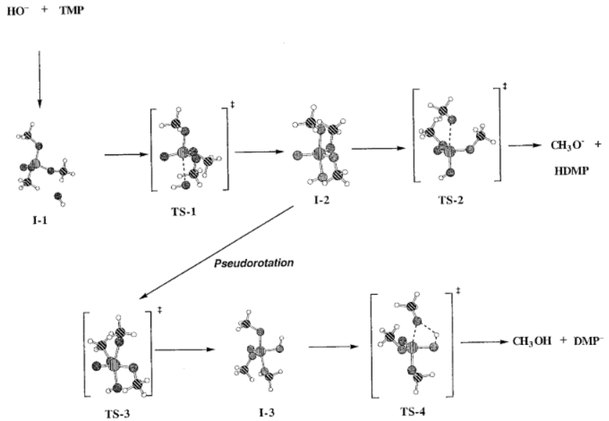
Table 8. Intra- and Intermolecular Contributions to the Rate Acceleration of Five-Membered Ring MEP Relative to Its Acyclic and Six-Membered Ring Analogs (All Energies Are Reported to 1 Decimal Place in kcal/mol)

	TMP−MEP			MPP−MEP		
a	*b,d*	*c,d*	*c,e*	*b,d*	*c,d*	*c,e*
$\Delta\Delta E^{\ddagger}$	−5.8	−3.8	−4.2	2.3	1.8	2.5
$\Delta\Delta\delta E^{\ddagger}$(T)	1.1	1.1	1.1	0.2	0.2	0.2
$\Delta(T\Delta S^{\ddagger})$	−1.3	−1.3	−1.3	−1.1	−1.1	−1.1
$\Delta\Delta G^{\ddagger}_{gas}$	−3.3	−1.3	−1.8	3.6	3.1	3.8
$\Delta\Delta G_s$(TS)	11.1	10.7	16.4	−1.2	3.1	3.5
$\Delta\Delta G_s$(GS)	1.3	1.3	2.5	0.3	0.3	0.7
$\Delta\Delta G^{\ddagger}_{sln}$	6.5	8.1	12.2	2.1	5.9	6.6

[a] Ground state to rate-limiting transition state. [b] Energies and free energies corresponding to gas-phase rate-limiting transition state. [c] Energies and free energies corresponding to relocated rate-limiting transition state in solution. [d] Solvation free energies computed using the continuum dielectric method. [e] Solvation free energies computed using combined PSGVB/DelPhi programs.

Appendix: Ab initio calculations for MEP vs. TMP or EPP

Scheme 1. Schematic Diagram Depicting Fully Optimized HF/6-31+G* Structures for the Reaction of $(OH)^-$ at the Phosphorus of TMP (TMP(P)) (Not All Transition States and Intermediates along the Pathways Are Depicted for the Sake of Clarity.)



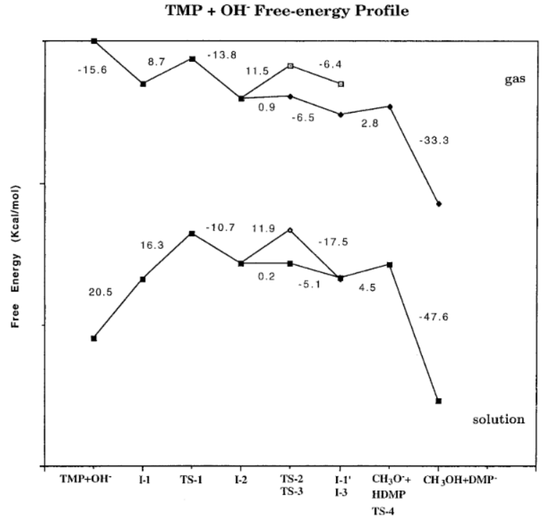
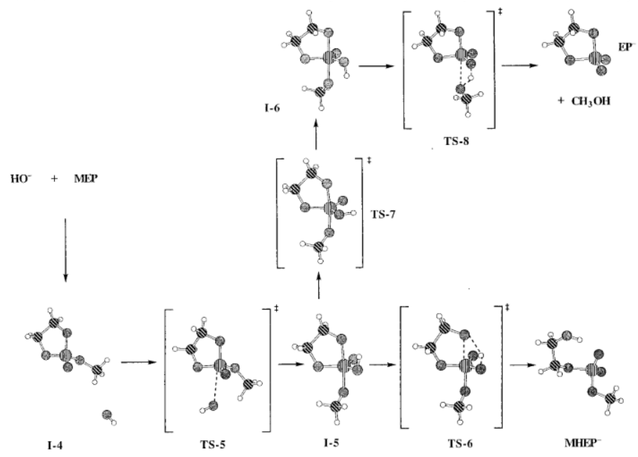


Figure 1. Relative MP2/6-31+G*//HF/6-31+G* activation free-energy profile for the gas-phase reaction of $(OH)^-$ with TMP (top) and the change in profile upon solvation (bottom). The zero of energy corresponds to the reactants at infinite separation. The numbers correspond to the free-energy differences of subsequent points. The free energy of the ion−dipole complex, $(CH_3O)^-\cdots$HDMP, which was not computed, was assumed to be similar to that of **I-1**.

Scheme 2. Schematic Diagram Depicting Fully Optimized HF/6-31+G* Structures for the Reaction of $(OH)^-$ at Phosphorus of MEP (MEP(P)) (Not All Transition States and Intermediates along the Pathways Are Depicted for the Sake of Clarity.)



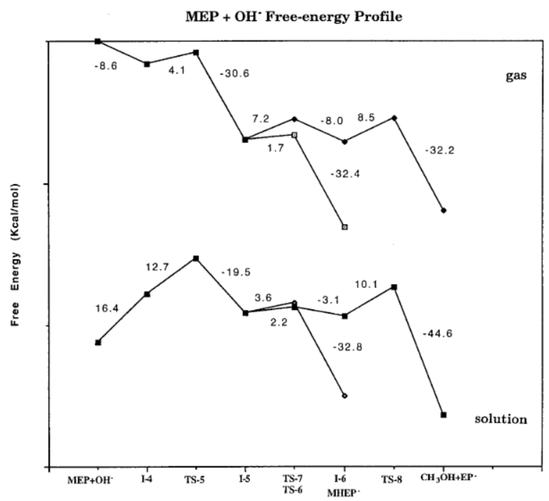
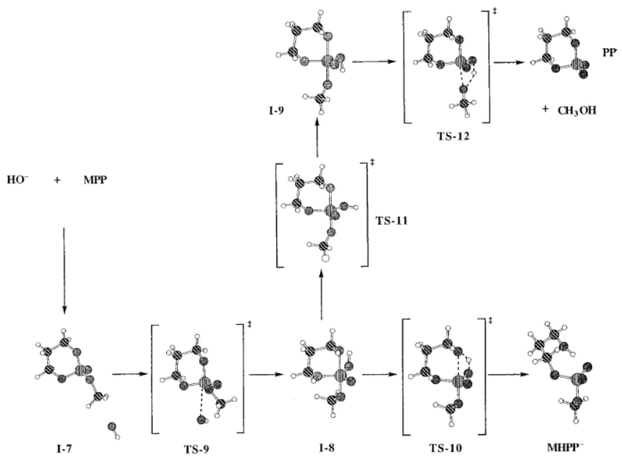


Figure 2. Relative MP2/6-31+G*//HF/6-31+G* activation free-energy profile for the gas-phase reaction of $(OH)^-$ with MEP (top) and the change in profile upon solvation (bottom). The zero of energy corresponds to the reactants at infinite separation. The numbers correspond to the free-energy differences of subsequent points.

Scheme 3. Schematic Diagram Depicting Fully Optimized HF/6-31+G* Structures for the Reaction of $(OH)^-$ at Phosphorus of MPP (MPP(P)) (Not All Transition States and Intermediates along the Pathways Are Depicted for the Sake of Clarity)



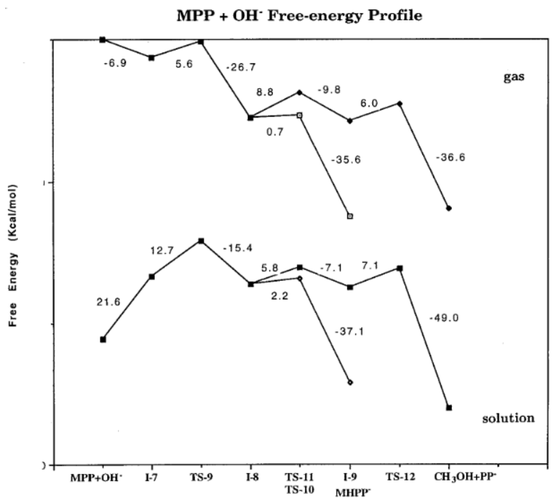


Figure 3. Relative MP2/6-31+G*//HF/6-31+G* activation free-energy profile for the gas-phase reaction of $(OH)^-$ with MPP (top) and the change in profile upon solvation (bottom). The zero of energy corresponds to the reactants at infinite separation. The numbers correspond to the free-energy differences of subsequent points.