

## Synthesis Cycle for a PS2 linkage by Using Tetrazole as Activator

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DNA Synthesizer: Expedite 8909  
 Scale: 1  $\mu$ mole  
 Thiophosphoramidite: 0.15 M in fresh anhydrous CH<sub>3</sub>CN (Glen Research, #40-4050-XX) containing 10% fresh anhydrous CH<sub>2</sub>Cl<sub>2</sub> (Aldrich, # 270997)  
 Activator: Tetrazole (0.45M)  
 Sulfurizing Reagent: 3-((N,N-dimethyl-aminomethylidene)amino)-3H-1,2,4-dithiazole-5-thione [DDTT, 0.05M solution in pyridine/CH<sub>3</sub>CN (3:2)] (Glen Research, # 40-4137-51)

### Consumed Major Chemical Volumes:

- Thiophosphoramidite: 125  $\mu$ L
- Activator: 250  $\mu$ L
- Sulfurizing Reagent: 3100  $\mu$ L

(The volumes of thiophosphoramidite and Activator are identical to normal phosphoramidite)

### Coupling yield:

- ~ 95% (tested based on HPLC analysis of 5'-X<sub>PS2</sub>T-3')

### Protocol Cycle (1.0 $\mu$ mole, Thiophosphoramidite at position 6)

\$Deblocking

No Change

\$Coupling (9 Min coupling time)

(0.15 M of Thiophosphoramidite is assembled at position 6)

\$Coupling					
1	/*Wsh	*/ PULSE	5	0	“Flush system with wash”
2	/*Act	*/ PULSE	5	0	“Flush system with Act”
23	/*6 + Act	*/ PULSE	5	0	“Monomer + Act to Column”
23	/*6 + Act	*/ PULSE	2	136	“Couple monomer”
2	/*Act	*/ PULSE	4	272	“Couple monomer”
1	/*Wsh	*/ PULSE	2	136	“Couple monomer”

1	/*Wsh	*/ PULSE	8	0	“Flush with wash”
1	/*Wsh	*/ PULSE	20	0	“ Wash”

### \$Sulfurizing

0.05 M DDTT from Glen Research					
17	/*Aux	*/ PULSE	20	5	“Aux to Column”
1	/*Wsh	*/ PULSE	6	60	“Flush with wash”
17	/*Aux	*/ PULSE	20	5	“Aux to Column”
1	/*Wsh	*/ PULSE	6	60	“Flush with wash”
17	/*Aux	*/ PULSE	20	5	“Aux to Column”
1	/*Wsh	*/ PULSE	6	60	“Flush with wash”
17	/*Aux	*/ PULSE	20	5	“Aux to Column”
1	/*Wsh	*/ PULSE	6	60	“Flush with wash”
17	/*Aux	*/ PULSE	20	5	“Aux to Column”
1	/*Wsh	*/ PULSE	6	60	“Flush with wash”
17	/*Aux	*/ PULSE	20	5	“Aux to Column”
1	/*Wsh	*/ PULSE	6	60	“Flush with wash”
17	/*Aux	*/ PULSE	20	5	“Aux to Column”
1	/*Wsh	*/ PULSE	6	60	“Flush with wash”
17	/*Aux	*/ PULSE	20	5	“Aux to Column”
1	/*Wsh	*/ PULSE	6	60	“Flush with wash”
12	/*Wsh A	*/PULSE	9	0	“Flush with wash”

### \$ Capping

No Change

### \$Deblocking:

No change

End of Cycle

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### Synthesis of chimeric DNA containing PS2 linkage(s)

It should be noted that that the Expedite 8909 oxidizing time as well as the oxidizing reagent volumes found in the manufacturer’s protocol are not sufficient to synthesize chimeric DNA containing PS2 linkage(s). A modified protocol that corrects these problems is attached for reference.

A modified protocol cycle for phosphodiester linkage(s)

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### \$Deblocking:

No change

\$Coupling:

No Change

\$Capping:

No Change

\$Oxidizing:

\$Oxidizing					
15	/*Ox	*/ PULSE	5	0	“ Flow system with”
15	/*Ox	*/ PULSE	10	30	“Ox to column”
12	/*Wash A	*/ PULSE	6	30	
12	/*Wash A	*/ PULSE	20	0	

\$Capping:

No Change

End of Cycle

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