AM Biotechnologies
THE THIOAPTAMER COMPANY

Synthesis Cycle for a PS2 linkage by Using DCI as Activator

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DNA Synthesizer: Expedite 8909
Scale: 1 µmole
Thiophosphoramidite: 0.15 M in fresh anhydrous CH_3CN (Glen Research, #40-4050-XX) containing 10% fresh anhydrous CH_2Cl_2 (Aldrich, # 270997)
Activator: DCI (>1M)
Sulfurizing Reagent: 3-((N,N-dimethyl-aminomethylidene)amino)-3H-1,2,4-dithiazole-5-thione [DDTT, 0.05M solution in pyridine/CH_3CN (3:2)] (Glen Research, # 40-4137-51)

Consumed Major Chemical Volumes:

- Thiophosphoramidite: 125 µL
- Activator: 250 µL
- Sulfurizing Reagent: 3100 µL

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

Coupling yield:

- ~ 95% (tested based on HPLC analysis of 5'-X_{PS2}T-3')

Protocol Cycle (1.0 µmole, Thiophosphoramidite at position 6)

$Deblocking

No Change

$Coupling (3 Min coupling time, DCI as activator)

(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	46	"Couple monomer"
2	/*Act	*/ PULSE	4	92	"Couple monomer"
1	/*Wsh	*/ PULSE	2	46	"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

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)

$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