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$_3$CN (Glen Research, #40-4050-XX) containing 10% fresh anhydrous CH$_2$Cl$_2$ (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$_3$CN (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)

<table>
<thead>
<tr>
<th>$Coupling$</th>
<th>$Wsh$</th>
<th>$Act$</th>
<th>$PS2$</th>
<th>$Act$</th>
<th>$PS2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>/Pulse 5</td>
<td>0</td>
<td>“Flush system with wash”</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>/Pulse 5</td>
<td>0</td>
<td>“Flush system with Act”</td>
<td></td>
<td></td>
</tr>
<tr>
<td>23</td>
<td>/Pulse 5</td>
<td>0</td>
<td>“Monomer + Act to Column”</td>
<td></td>
<td></td>
</tr>
<tr>
<td>23</td>
<td>/Pulse 2</td>
<td>46</td>
<td>“Couple monomer”</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>/Pulse 4</td>
<td>92</td>
<td>“Couple monomer”</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>/Pulse 2</td>
<td>46</td>
<td>“Couple monomer”</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Synthesis of chimeric DNA containing PS2 linkage(s)

It should be noted 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

End of Cycle
No change

$Coupling:

No Change

$Capping:

No Change

$Oxidizing:

<table>
<thead>
<tr>
<th>Time</th>
<th>Condition</th>
<th>Flow System with</th>
<th>Ox to column</th>
</tr>
</thead>
</table>
| 15   | Ox        | 5
| 15   | Ox        | 10
| 12   | Wash A    | 6
| 12   | Wash A    | 20

$Capping:

No Change

End of Cycle