Supporting Information

New, Stronger Nucleophiles for Nucleic Acid-Templated Chemistry: Synthesis and Application in Fluorescence Detection of Cellular RNA

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Synthesis cycle for on-column addition of dithiaphospholane.*

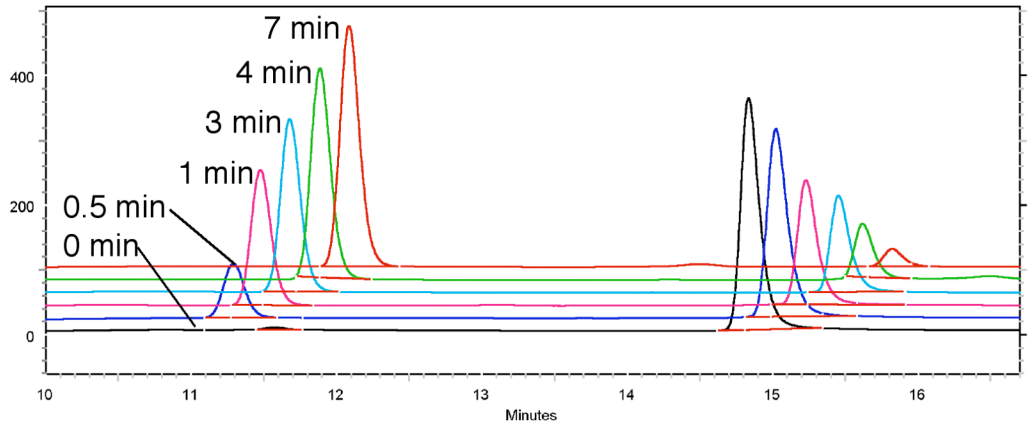
Step	Function	Time (s)
1	Begin	
2	CH_3CN to waste	6
3	CH_3CN to column	13
4	Reverse flush	10
5	Block flush	4
6	Phos prep	5
7	Block vent	2
8	Tetrazole to waste	1.7
9	Base (iPr_2N-phospholane) + tetrazole to column	2.5
10	Tetrazole to column	1
11	Base (iPr_2N-phospholane) + tetrazole to column	3
12	Push to column	
13	CH_3CN to waste	10
14	Block flush	4
15	Wait	120
16	Phos prep	5
17	Block vent	2
18	Tetrazole to waste	1.7
19	Base (iPr_2N-phospholane) + tetrazole to column	2.5
20	Tetrazole to column	1
21	Base (iPr_2N-phospholane) + tetrazole to column	3
22	Push to column	
23	CH_3CN to waste	10
24	Block flush	4
25	Wait	120
26	CH_3CN to column	30
27	Reverse flush	10
28	CH_3CN to column	15
29	Reverse flush	10
30	Block flush	4
31	Prep 10 (1:1 CS_2/pyridine)	10
32	10 to column	10
33	Reverse flush	15
34	Phos prep	10
35	5 (Sulfur in 1:1 CS_2/pyridine) to column	15
36	10 to waste	7
37	Block flush	4
38	Wait	180
39	5 (Sulfur in 1:1 CS_2/pyridine) to column	15
40	10 to waste	7
41	Block flush	4
42	Wait	180
43	10 to column	25
44	Reverse flush	10

45	10 to column	15
46	Reverse flush	10
47	10 to column	15
48	Reverse flush	10
49	Block flush	4
50	CH_3CN to column	15
51	Reverse flush	10
52	End	

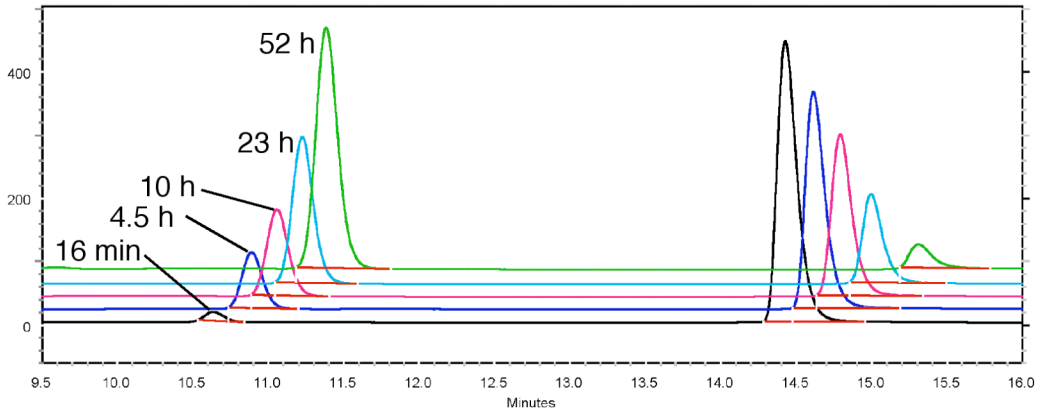
*Written for ABI 392 and 394 DNA synthesizers.

HPLC analysis of dT-PS2 and dT-PS3 stability

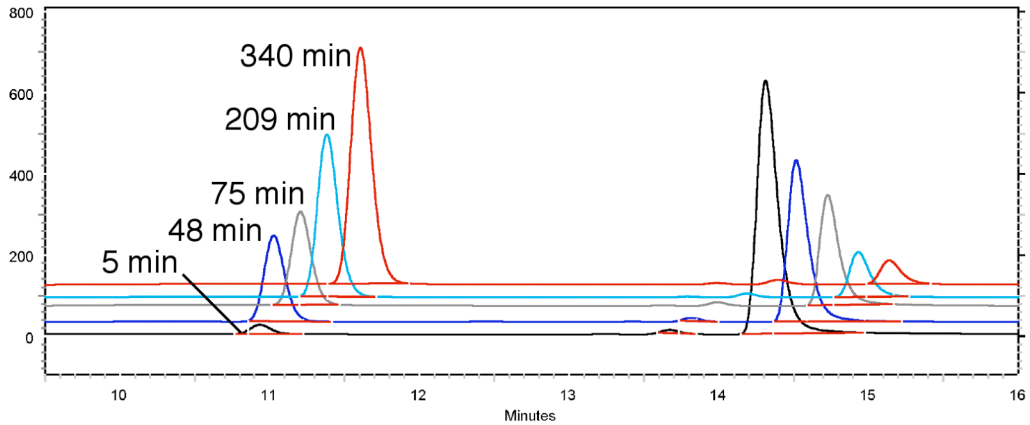
All samples used in stability studies were stored frozen in pH 9.0 Tris buffer (20 mM) at -80 °C until the start of each reaction. pH 5.0 and 7.0 stability studies were initiated at Time=0 by addition of the dT-PSx sample to pH-adjusted incubation buffers. Aliquots removed at early time points (<10min) were added to a pH 9.0 buffer before HPLC analysis to prevent further decomposition; later time points were injected directly onto the HPLC. Samples were analyzed on a Waters X-terra MS C18 column using a 0-14% gradient of methanol in 0.1 M TEAA (pH 9.0) over 12 minutes, followed by a 5-minute rinse step using 40% methanol.



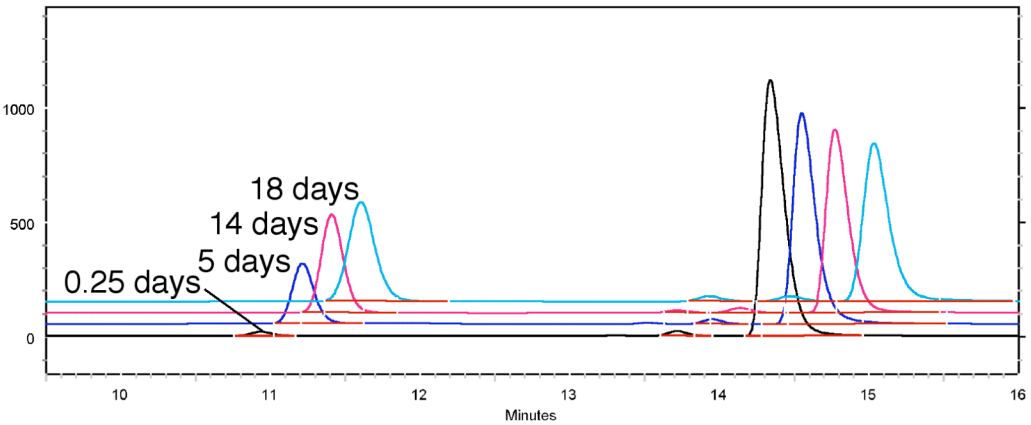
HPLC chromatograms of dT-PS3 at pH 5.0, 22ºC, in 20 mM Tris acetate. Early eluting product (11.3 min.) is dT-OH; late eluting product (14.8 min.) is dT-PS3 starting material.



HPLC chromatograms of dT-PS3 at pH 7.0, 22ºC, in 20 mM Tris acetate. Early eluting product (10.7 min.) is dT-OH; late eluting product (14.5 min.) is dT-PS3 starting material.



HPLC chromatograms of dT-PS2 at pH 5.0, 22ºC, in 20 mM Tris acetate. Early eluting product (11 min.) is dT-OH; late eluting product (14.4 min.) is dT-PS2 starting material.



HPLC chromatograms of dT-PS2 at pH 7.0, 22ºC, in 20 mM Tris acetate. Early eluting product (11 min.) is dT-OH; late eluting product (14.4 min.) is dT-PS2 starting material.