Supporting Information For

Synthetic Oligodeoxynucleotide Purification by Polymerization of Failure Sequences

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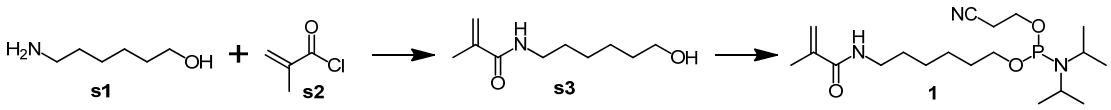
General experimental

ODNs were synthesized on a standard ABI 394 solid phase synthesizer. MALDI-TOF mass spectra were obtained on a Shimadzu Biotech Axima CFRplus spectrometer. HPLC was performed on a JASCO LC-2000Plus System: pump, PU-2089Plus Quaternary Gradient; detector UV-2075Plus. A C-18 reverse phase analytical column (5 μm diameter, 100 Å, 250 × 3.20 mm) was used. Solvent A: 0.1 M triethylammonium acetate, 5% acetonitrile. Solvent B: 90% acetonitrile. All profiles were generated by detection of absorbance of ODN at 260 nm using the linear gradient solvent system: solvent B (0%-45%) in solvent A over 60 min followed by solvent B (45%-100%) in solvent A over 20 min at a flow rate of 0.5 mL/min. Succinic ester linked DMTr-dT-lcaa-CPG (pore size 1000 Å) and 5'-DMTr, 2-cyanoethyl phosphoramidites acetyl-dC, Pac-dA, 4-isopropyl-Pac-dG and dT were purchased from Glen Research, Inc. D-Salt™ dextran desalting column (5K MWCO) was purchased from Pierce Biotechnology, Inc.

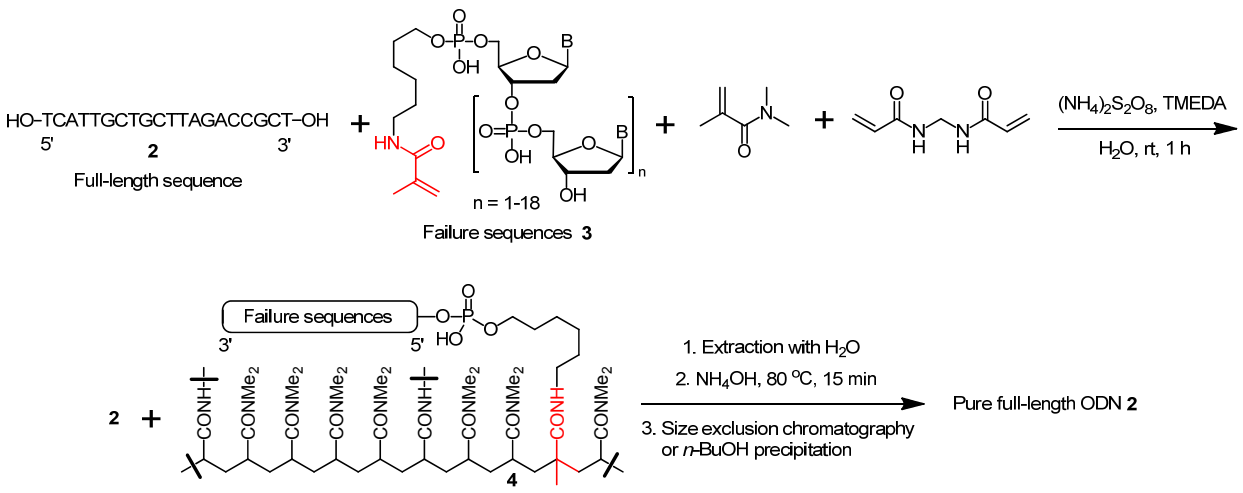
Synthesis of phosphoramidite 1



Compound **1** is known.[1] We used a different method to prepare **s3**, and only this step is described here. Compound **s1** (5.0 g, 42.7 mmol), saturated Na_2CO_3 solution (50 mL) and CH_2Cl_2 (150 mL) were charged into a round bottom flask, and cooled to 0 ºC. To the solution was added the solution of **s2** (4.46g, 42.7 mmol) in CH_2Cl_2 (50 mL) dropwise with efficient stirring via an addition funnel. After addition, the mixture was stirred at rt for 3 h, and then transferred into a separation funnel. The organic layer was separated. The aqueous phase was extracted with CH_2Cl_2 for three times. The combined organic phase was dried over anhydrous Na_2SO_4, and filtered. The solution was concentrated to give a sticky liquid (not completely dry). Ether was added until white solids appeared, which was re-dissolved by adding CH_2Cl_2. To make the solution more dilute, hexane and more CH_2Cl_2 were added. The solution was then put in a freezer (-20 ºC) for 12 h. Compound **s3** was obtained as a colorless crystal: 7.33 g, 93% yield. If the compound melts at rt, a second crystallization from CH_2Cl_2/hexane may be desirable.

Synthesis of ODN 2 and its purification by polymerization of failure sequences

Synthesis of ODN 2. ODN **2** was synthesized on a standard ABI 394 solid phase synthesizer using standard phosphoramidite chemistry under UltraMild conditions on controlled pore glass (CPG, pore size 1000 Å) on a 0.2 μmol scale. The succinyl ester linkage was used to anchor the ODN to CPG. The phosphoramidite monomers used were Pac-dA-CE, Ac-dC-CE, *i*-Pr-Pac-dG-CE and dT-CE. A 0.2 M solution of the polymerizable capping phosphoramidite **1** in acetonitrile was placed on the 5th bottle position, which is normally used for incorporating an additional base into ODN. The two bottles normally used to supply Ac_2O capping reagents were empty. The synthesis was accomplished using the synthetic cycle shown in the *ODN synthetic cycle using polymerizable phosphoramidite as capping agent* section of this supporting information. In this synthesis, the capping failure sequences step was achieved using the polymerizable phosphoramidite **1** with 1*H*-tetrazole as the activator. The activator was from the same bottle that provided 1*H*-tetrazole for the coupling steps. To ensure complete capping, **1** and tetrazole were delivered to the synthesis column four times instead of two times normally used for standard nucleobase coupling (we found that capping two times were not enough for complete capping under the conditions we used. This can be seen from HPLC trace **h** in the *larger images of HPLC profiles* section of this supporting information, where the small peaks before the major full-length peak are un-capped failure sequences). Between each delivery, a waiting time of 15 seconds was applied. The oxidation of the phosphite triesters between the capping agent and failure sequences was carried out for three times. In the last synthetic cycle, the DMTr group was removed. Cleavage and deprotection were carried out on the synthesizer with concentrated NH_4OH (900 min × 4) at rt. The ODN solution was distributed equally into 4 Eppendorf tubes (1.5 mL), and dried in a SpeedVac, separately (each portion contained ~50 nmol ODN, P_1-P_4). P_1 was dissolved in 150 μL water, 20 μL (~6.67 nmol) was injected into RP HPLC to generate trace **a** (Figure 1). The full-length sequence appeared at ~19 minutes. The failure sequences were at ~20 minutes. The small peaks at around 10, 28, and 56 minutes were probably due to the small molecules from protecting groups. The very small peaks, which could be hardly seen in **a** at the left of the full-length sequence, might be resulted from un-capped failure sequences, deletion sequences, or damaged sequences.



Polymerization of failure sequences. The remaining 130 μL solution of P_1 was transferred into a 2-necked round bottom flask. The Eppendorf tube was washed with water (50 μL × 3); the washes were also placed into the same flask. To the flask was added the pre-formed polymerization solution [250 μL; dimethylacrylamide 1.69 M, *N*,*N'*-methylenebis(acrylamide) 16.9 mM; the solution could be stored at -20 ˚C in dark for 1 month]. The flask was flushed with nitrogen for 2 min with gentle stirring. $(NH_4)S_2O_8$ (10%, 5 μL) and *N*,*N*,*N'*,*N'*-tetramethylethylenediamine (TMEDA, 5 μL) were added sequentially via pipettes under positive nitrogen pressure. The solution was stirred gently under nitrogen at rt. A gel, which was the ODN failure sequences-polyacrylamide conjugate **4**, was formed within 30 min. The gel was allowed to stand for another 30 min to ensure completion of polymerization.

Extraction of full-length sequence. To the gel, which was broken into several pieces, was added water (200 μL). The mixture was stirred gently (shaking should be better) at rt for 3 h. The supernatant was transferred into an Eppendorf tube. The gel was further extracted with water for 2 times (200 μL, rt, 12 h; 200 μL, rt, 3). The supernatants were combined and evaporated into dryness. The ODN was dissolved into 130 μL water; 20 μL was injected into HPLC to generate trace **b**. As shown, the failure sequences were removed, but the full-length sequence appeared as 4 peaks. The small peaks resulted from small molecules from protecting groups also remained as expected.

Size exclusion chromatography to remove small molecules. A 10 mL D-Salt™ dextran desalting column (5K MWCO) was used. The column was first washed with water (20 mL). The

remaining 110 μL solution of ODN **2** was loaded to the top of the column. The Eppendorf tube was washed with water (100 μL × 3), and the washes were also loaded to the column. The column was washed with 1.59 mL water. This first 2 mL eluent did not contain any ODN. The elution was continued and the next 5 mL was collected and evaporated to dryness. The residue was dissolved in 110 μL water, 20 μL was injected into HPLC to generate trace **c**. As shown, the small molecules from protecting groups were removed. The 4 peaks at around 19 min were merged into 3. The desalting column was recovered by washing with water (20 mL), and was stored in 0.02% NaN_3 solution.

Treating with concentrated NH_4OH. The remaining 90 μL solution of ODN **2** was evaporated to dryness in an Eppendorf tube. Concentrated NH_4OH (300 μL) was added. The solution was heated to 80 ºC for 15 min in a sand bath. After evaporation to dryness, the residue was dissolved into 90 μL water, 20 μL was injected into HPLC to generate trace **d**. As shown, only one peak is observed, and the ODN is pure. The recovery yield of the purification process (polymerization, extraction, size exclusion chromatography and NH_4OH treatment) was determined to be 83% by comparing the area of the peak in trace **d** with that in trace **a** at ~19 min.

Identification of ODN 2. The authentic ODN of **2** was synthesized using standard phosphoramidite chemistry on a 1 μmol scale and purified with trityl-on RP HPLC at The Midland Certified Reagent Company, Inc. (Midland, TX, USA). MALDI-TOF spectrum of the authentic ODN was also obtained at the company showing correct molecular weight: calcd for $[M - H]^-$ 6057, found 6060. At Michigan Tech, the authentic sample was divided equally into 10 portions (each portion contains ~100 nmol ODN assuming the yield for the synthesis and purification was 100%). One portion was dissolved in 150 μL water, 20 μL was injected into RP HPLC to generate trace **f** (Figure 1). To compare ODN **2** synthesized in our lab and purified using our catching failure sequences by polymerization technique with the authentic sample, 10 μL of the solution used to generate trace **d** and 10 μL of the solution used to generate trace **f** were mixed, and injected into RP HPLC to generate trace **e** (Figure 1). A single peak was observed showing the two were identical. ODN **2** was also characterized with MALDI-TOF: calcd for $[M - H]^-$ 6057, found: 6057. The spectrum is attached in the *MALDI-TOF mass*

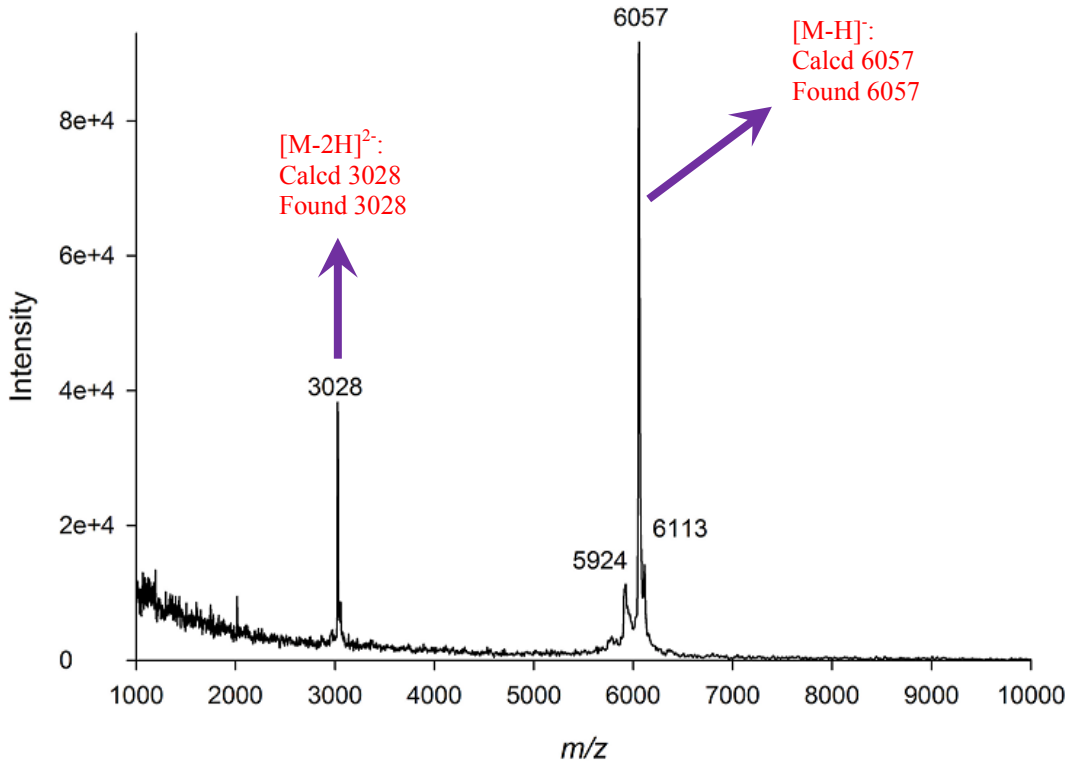
*spectrum of ODN **2** purified by polymerization of failure sequences approach* section of this supporting information.

ODN purification by polymerization of failure sequences and n-BuOH precipitation. The ODN **2** (P_2, ~50 nmol) was dissolved into 150 μL water, 20 μL was injected into HPLC, which gave a trace exactly the same as **a**. The remaining 130 μL ODN solution was subjected into the procedure of polymerization of failure sequences and extraction of full-length sequence as described above. The size exclusion chromatography step was not performed. To the full-length sequence, which contained 4 ODNs and small organic molecules from protecting groups, was added concentrated NH_4OH (100 μL). The solution was vortexed shortly and then heated to 80 ºC for 30 min. This converted the modified ODNs to the un-modified one. After cooling to rt, *n*-BuOH (1 mL) was added. The mixture was vortexed for 30 sec and then centrifuged at 14.5K for 5 min. The supernatant was removed. The residue was re-dissolved into 50 μL water, 500 μL *n*-BuOH was added. Votexed and centrifuged again, and the supernatant was removed. This removed the small organic molecules resulted from deprotection. The ODN was dissolved in 130 μL water, 20 μL was injected into HPLC to generate trace **g**. As shown, the ODN is pure. The recovery yield of the procedure (polymerization, extraction, NH_4OH treatment and *n*-BuOH precipitation) was determined to be 85% by comparing the area of the peak in trace **g** with that in trace **a** at ~19 min.

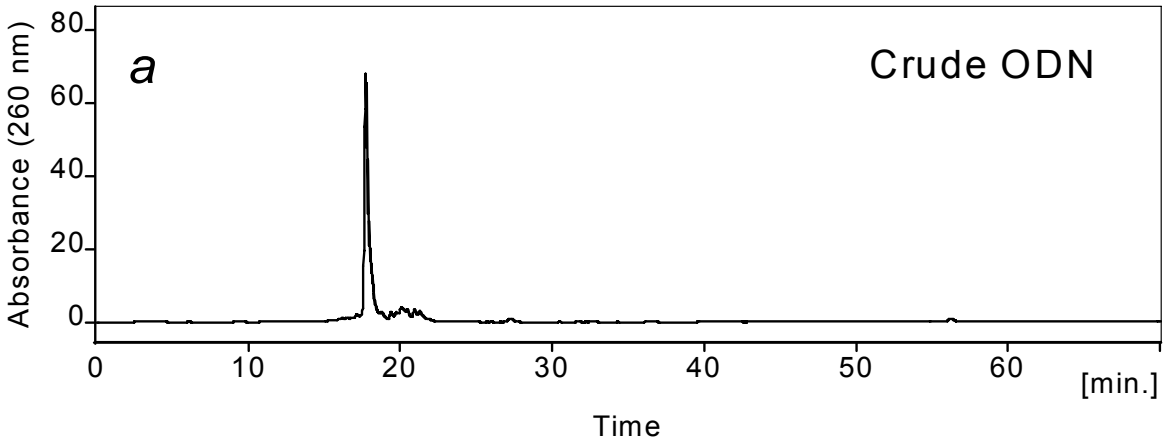
Reference

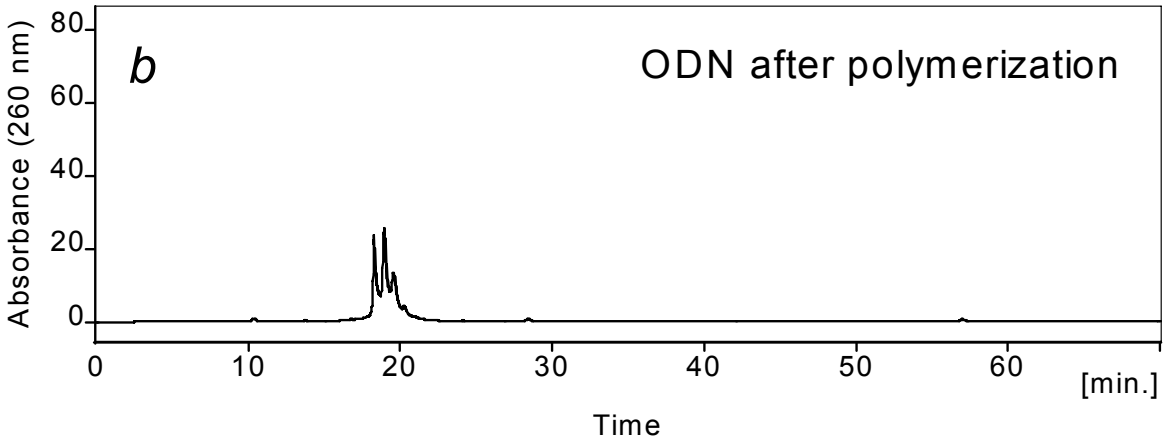
1. Z. Zhu, C. C. Wu, H. P. Liu, Y. Zou, X. L. Zhang, H. Z. Kang, C. J. Yang and W. H. Tan, *Angew. Chem. Int. Edit.*, 2010, **49**, 1052-1056.

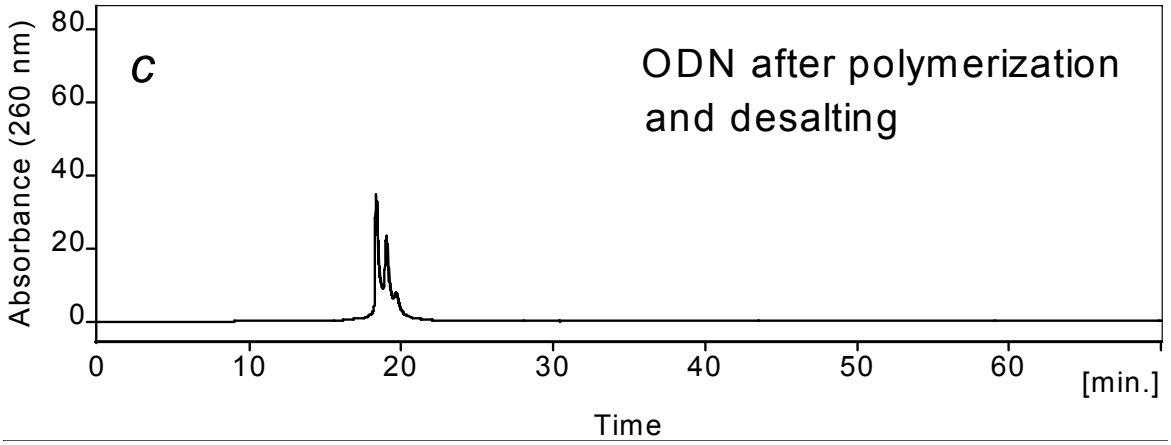
MALDI-TOF mass spectrum of ODN 2 purified by polymerization of failure sequences

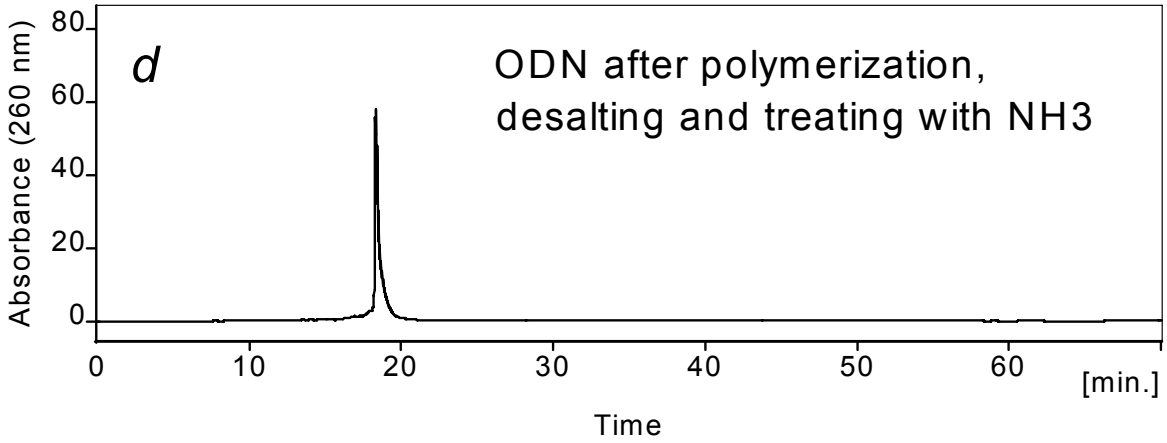


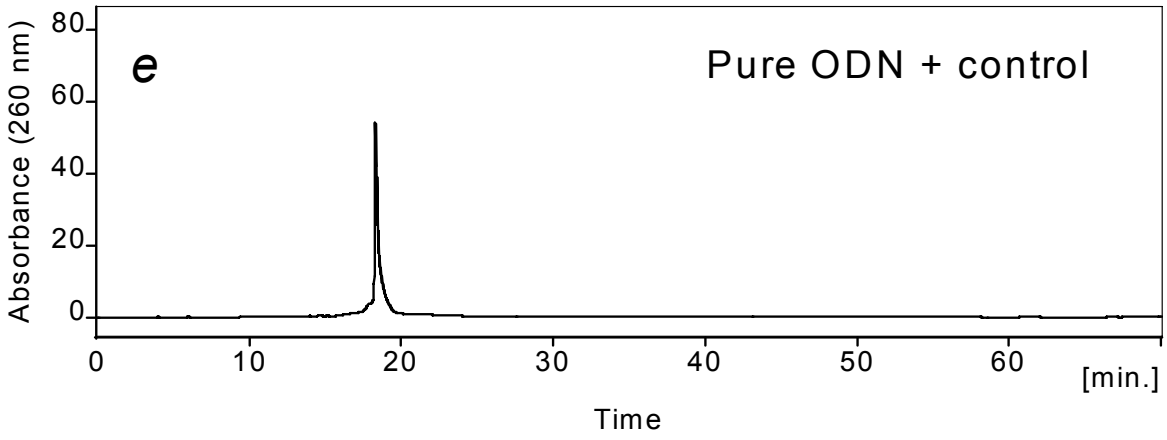
Larger images of HPLC profiles

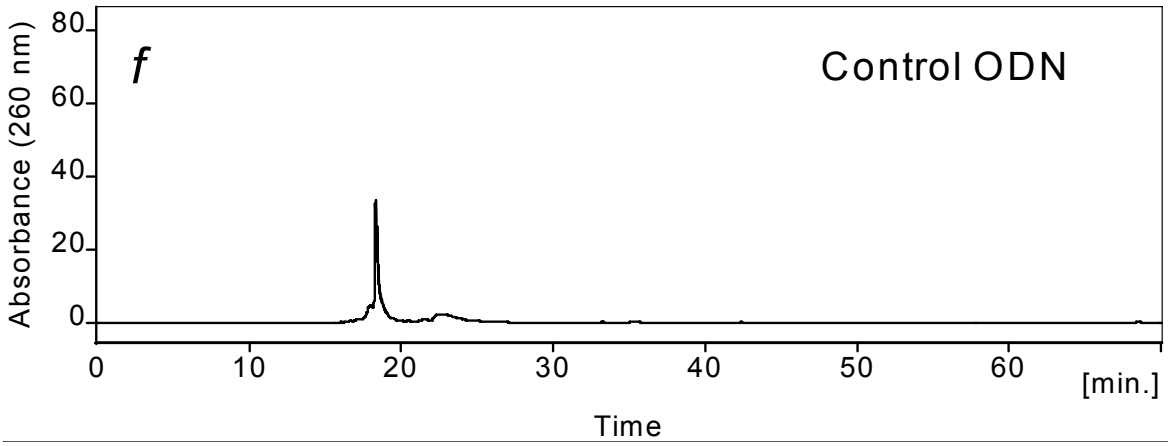


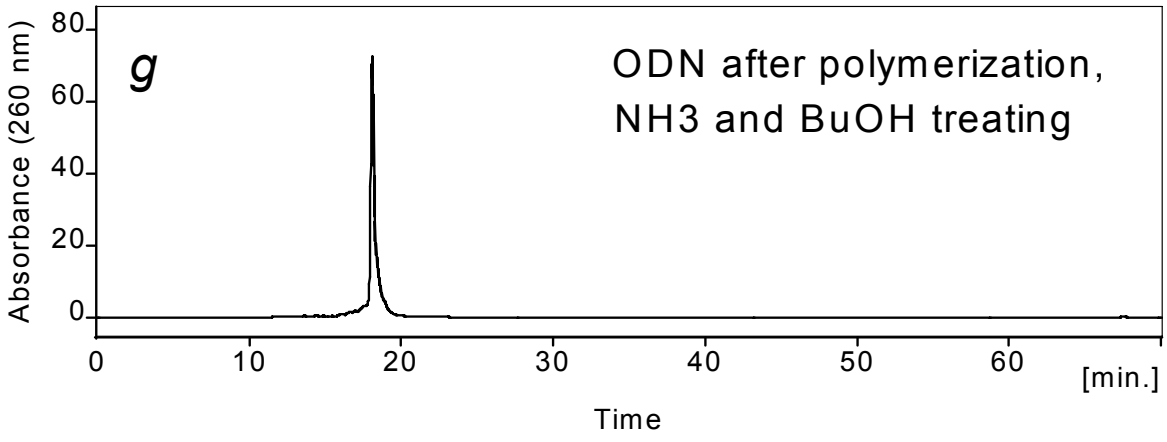


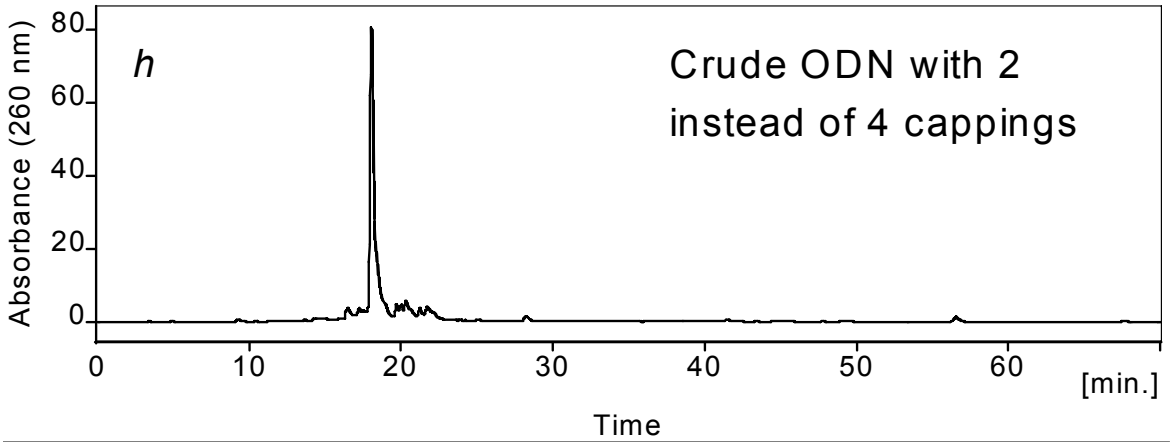

c
ODN after polymerization
and desalting
Absorbance (260 nm)
80
60
40
20
0
0
10
20
30
40
50
60
[min.]
Time


d
ODN after polymerization,
desalting and treating with NH3
Absorbance (260 nm)
80
60
40
20
0
0
10
20
30
40
50
60
[min.]
Time


e
Pure ODN + control
Absorbance (260 nm)
80
60
40
20
0
0
10
20
30
40
50
60
[min.]
Time


f
Control ODN
Absorbance (260 nm)
80
60
40
20
0
0
10
20
30
40
50
60
[min.]
Time


g
ODN after polymerization,
NH3 and BuOH treating
Absorbance (260 nm)
80
60
40
20
0
0
10
20
30
40
50
60
[min.]
Time


h
Crude ODN with 2
instead of 4 cappings
Absorbance (260 nm)
80
60
40
20
0
0
10
20
30
40
50
60
[min.]
Time

ODN synthetic cycle using polymerizable phosphoramidite as capping agent

ODN synthetic cycle using polymerizable phosphoramidite **1** as the capping agent
Synthesizer: standard ABI 394 solid phase synthesizer; 4-column 8-base instrument
Polymerizable capping agent: 0.2 M solution of **1** in acetonitrile, placed at the bottle 5 position
The bottles for normal Ac_2O capping agents are empty
Activator for the capping phosphoramidite: from the same bottle for the coupling step
Synthesis scale: 0.2 μmol
Column used: column 2

Step number	Function number	Function name	Step time
1.	106	Begin	
2.	64	18 To waste	3.0
3.	42	18 To column	10.0
4.	2	Reverse flush	8.0
5.	1	Block flush	4.0
6.	101	Phos Prep	3.0
7.	142	Column 2 on	
8.	64	18 To waste	4.0
9.	1	Block flush	3.0
10.	111	Block vent	2.0
11.	58	Tet to waste	1.7
12.	33	B+Tet to column	2.0
13.	34	Tet to column	1.0
14.	33	B+Tet to column	1.5
15.	43	Push to column	
16.	143	Column 2 off	
17.	103	wait	25.0
18.	64	18 To waste	4.0
19.	2	Reverse flush	5.0
20.	1	Block flush	3.0
21.	41	15 To column	8.0
22.	64	18 To waste	4.0
23.	1	Block flush	3.0
24.	103	Wait	15.0

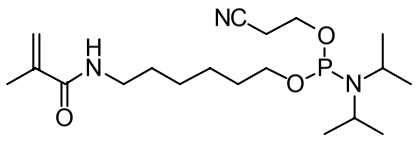
25.	42	18 To column	10.0
26.	4	Flush to waste	4.0
27.	42	18 To column	10.0
28.	2	Reverse flush	5.0
29.	1	Block flush	3.0
30.	142	Column 2 on	
31.	64	18 To waste	4.0
32.	1	Block flush	3.0
33.	111	Block vent	2.0
34.	58	Tet to waste	1.7
35.	35	5+Tet to column	2.5
36.	103	Wait	15.0
37.	34	Tet to column	1.0
38.	35	5+Tet to column	1.5
39.	103	Wait	15.0
40.	34	Tet to column	1.0
41.	35	5+Tet to column	1.5
42.	103	Wait	15.0
43.	34	Tet to column	1.0
44.	35	5+Tet to column	1.5
45.	103	Wait	15.0
46.	43	Push to column	
47.	143	Column 2 off	
48.	103	Wait	30.0
49.	142	Column 2 on	
50.	64	18 To waste	4.0
51.	2	Reverse flush	5.0
52.	1	Block flush	3.0
53.	41	15 To column	12.0
54.	103	Wait	22.0
55.	41	15 To column	5.0
56.	103	Wait	15.0
57.	41	15 To column	5.0
58.	64	18 To waste	4.0
59.	1	Block flush	3.0

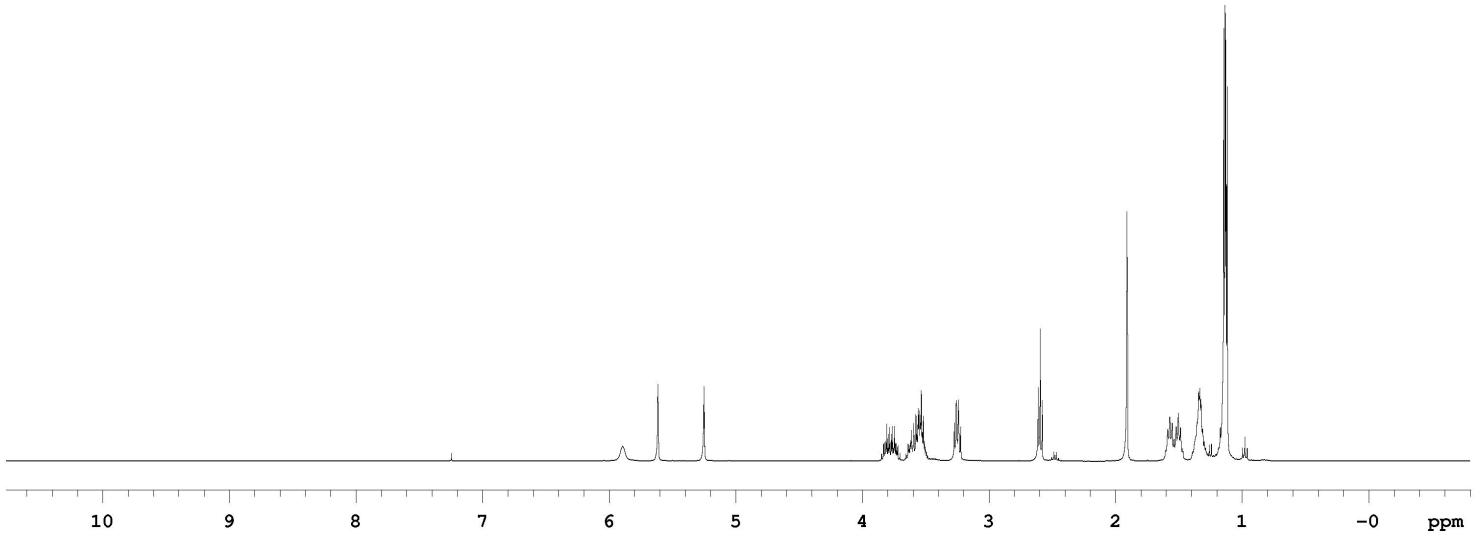
60.	103	Wait	15.0
61.	42	18 To column	10.0
62.	4	Flush to waste	4.0
63.	42	18 To column	10.0
64.	2	Reverse flush	5.0
65.	1	Block flush	3.0
66.	105	Start detrityl	
67.	64	18 To waste	4.0
68.	42	18 To column	10.0
69.	2	Reverse flush	5.0
70.	1	Block flush	3.0
71.	167	If monitoring	
72.	44	19 To column	25.0
73.	40	14 To column	3.0
74.	135	Monitor triyls	
75.	40	14 To column	25.0
76.	136	Monitor noise	
77.	40	14 To column	10.0
78.	137	Stop monitor	
79.	42	18 To column	10.0
80.	2	Reverse flush	8.0
81.	168	If not monitoring	
82.	40	14 To column	6.0
83.	3	Trityl flush	5.0
84.	40	14 To column	6.0
85.	103	Wait	5.0
86.	3	Trityl flush	5.0
87.	40	14 To column	6.0
88.	103	Wait	5.0
89.	3	Trityl flush	5.0
90.	40	14 To column	6.0
91.	103	Wait	5.0
92.	3	Trityl flush	5.0
93.	42	18 To column	10.0
94.	3	Trityl flush	8.0

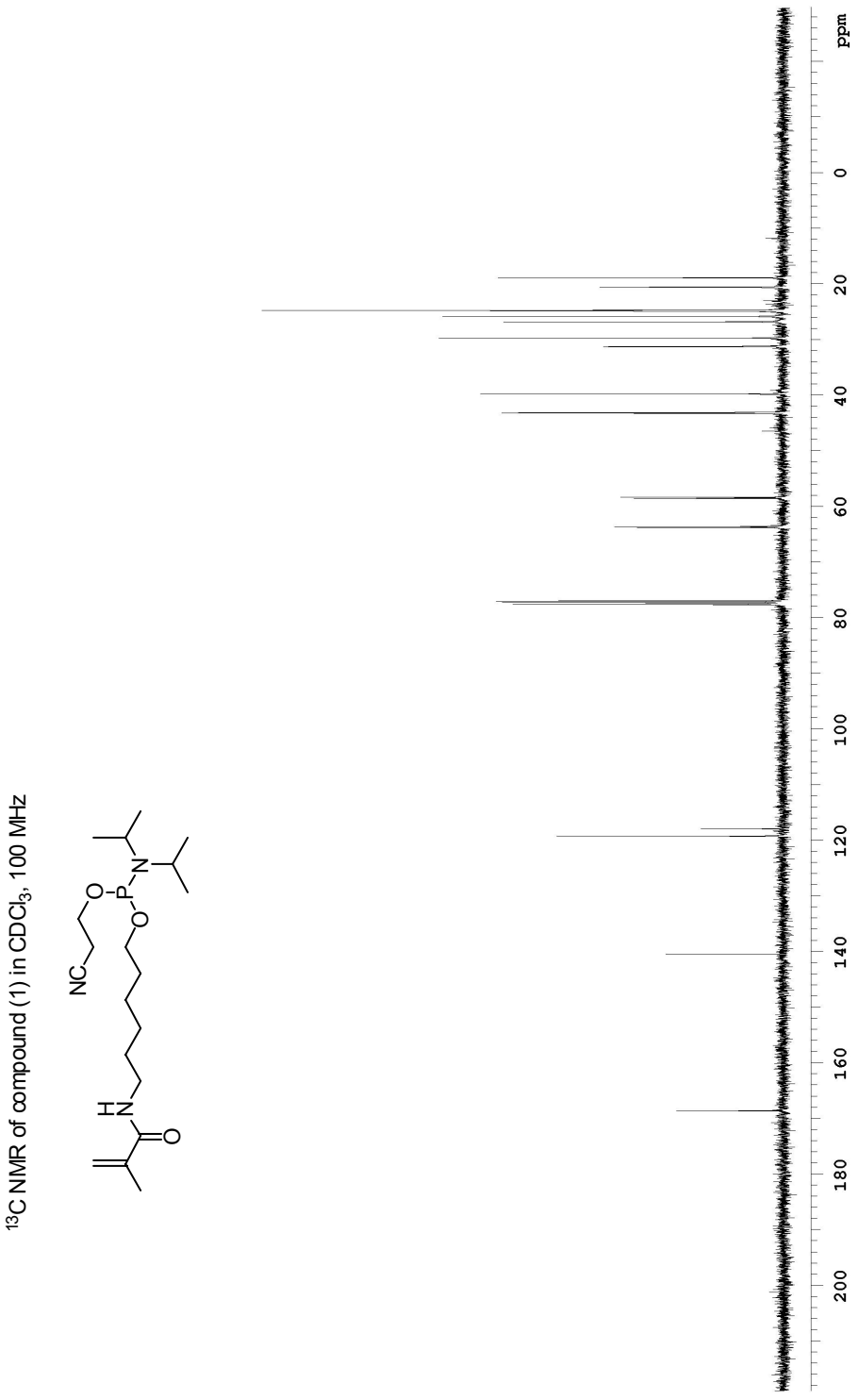
95.	169	End monitoring	
96.	42	18 To column	8.0
97.	2	Reverse flush	5.0
98.	1	Block flush	4.0
99.	107	End	

Note: Bottle 18—acetonitrile; bottle 5—capping phosphoramidite **1**; bottle 14—detritylation solution; bottle 15—oxidation solution; bottle 19—dichloromethane.

^{1}H NMR of compound (1) in $CDCl_3$, 400 MHz







^{31}P NMR of compound (1) in $CDCl_3$, 160 MHz