Electronic Supplementary Information

Synthetic Oligodeoxynucleotide Purification by Capping Failure Sequences with a Methacrylamide Phosphoramidite Followed by Polymerization†

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General methods: Reagents and solvents commercially available were used as received unless otherwise noted. CH_2Cl_2 was distilled over CaH_2 under a nitrogen atmosphere. Reactions were carried out under nitrogen in oven-dried glassware. Thin layer chromatography (TLC) was performed using TLC plates with silica gel 60F-254 over glass support, 0.25 µm thickness. Flash column chromatography was carried out using silica gel with particle size of 32 – 63 µm. ^{1}H, ^{13}C and ^{31}P NMR spectra were measured at 400, 100 and 162 MHz, respectively. HRMS was obtained using a TOF mass analyzer. RP HPLC: A C-18 analytical column (5 µm diameter, 100 Å pore size, 250 × 3.2 mm dimension) was used. Solvent A: 0.1 M triethylammonium acetate, 5% acetonitrile. Solvent B: 90 % acetonitrile. Profiles were generated by detection of absorbance of ODN at 260 nm using the following gradient system unless indicated otherwise: 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. Ion-exchange HPLC: An analytical Gen-Pak FAX (100 × 4.6 mm) column was used. Solvent A: 25 mM Tris/Cl, 1 mM EDTA, pH 8.0. Solvent B: 25 mM Tris/Cl 1 mM EDTA, 1.0 M NaCl, pH 8.0. The profile was generated by detection of absorbance of ODN at 260 nm using the gradient system: solvent B (10%-75%) in solvent A over 30 min followed by solvent B (75%) in solvent A for 30 min at a flow rate of 0.5 mL/min. Succinic ester linked DMTr-dT-lcaa-CPG (1000 Å pore size) and 5′-DMTr, 2-cyanoethyl phosphoramidites Pac-dA, benzoyl-dA, acetyl-dC, 4-isopropyl-Pac-dG, isobutyryl-dG, dT and other commonly used reagents for solid phase ODN synthesis were from commercial sources. The polymerization solution with a cross-linking ratio of 1:25 was used in all cases unless otherwise noted. The solution could be stored in a freezer at -20 ºC in dark for one month without affecting the results of catching by polymerization purification. High resolution MS and elemental analysis data for phosphoramidites were not collected due to their sensitivity to air and moisture.

Effect of one-month old capping agent on purification

For the catching by polymerization purification technology to be practically useful, it is important that capping phosphoramidites stored for a reasonable time have a similar capping efficiency as freshly prepared ones. For this reason, we stored compound **11** in a freezer under a nitrogen atmosphere at -20 ºC. After one month, its acetonitrile solution was prepared and used for the synthesis ODN **20** for capping failure sequences. The synthesis procedure with shortened

capping time (Cycle 2) was used. The crude ODN, which is labeled as **20**(c**11**o), was purified with the catching failure sequences by polymerization method. RP HPLC analysis (see this ESI) indicated that the purification results were the same as with freshly prepared capping agents. The recovery yield of the purification process was estimated to be 87%. The purity is 92%.

Effect of radical scavenger on purification

For one time, when pure phosphoramidite **10**, which was a thick oil, was dried under high vacuum at room temperature overnight, it became a gel. The reason is that under such conditions, the amount of oxygen, which serves as a radical scavenger, was significantly reduced, and radical polymerization occurred. To avoid premature radical polymerization, since then all capping phosphoramidites (**10**-**13**) were dried over drierite in a dessicator under a relatively low vacuum (achieved using an oil pump for an appropriate time and then close the dessicator) at -20 ºC in a freezer overnight. With the new drying method, we never met premature polymerization problem again. In the case of the solution of the methacrylamide phosphoramidites (**10**-**13**) in acetonitrile, premature polymerization never occurred in our hands. They are stable for at least three days on a synthesizer or in a freezer (-20 ºC) under nitrogen. Despite these favorable observations, potential users of the technology may still be interested to know if the capping steps during ODN synthesis are compatible with radical polymerization inhibitors in the capping solution. To clarify, ODN **20** was synthesized using the procedure with shortened capping time (Cycle 2). The solution of **11** (0.15 M) in acetonitrile with 500 ppm 2,6-di-tert-4-methylphenol was used for capping. The crude ODN was purified by catching failure sequences in the usual way. RP HPLC analysis (see this ESI) revealed that the ODN, which is labeled as **20**(c**11**i), was pure indicating that the radical scavenger studied did not adversely affect the purification results. The recovery yield of the purification process was estimated to be 97%. The purity is 98%.

Effect of harsher deprotecting conditions on purification

In our studies so far, the *exo*-amino groups on adenine and guanine were protected with phenoxyacetyl (Pac) and isopropyl-phenoxyacetyl (*i* -Pr-Pac) groups, respectively. These groups along with the acetyl group on cytosine were removed with concentrated NH_4OH at room temperature after synthesis. This protecting strategy has the advantage of avoiding deprotection under ammonia pressure in a sealed vial at elevated temperature. In the less expensive and more commonly used ODN synthesis methods, adenine and guanine are protected with benzoyl and isobutyryl groups, respectively, and deprotection are usually achieved with concentrated NH_4OH at elevated temperature. Previously, we showed that the harsher deprotecting conditions were compatible with the catching full-length sequences by polymerization ODN purification technology (Y. Yuan et al, *RSC Adv.*, 2012, **2**, 2803). Here, we show that the conditions are also compatible with the catching failure sequences method. The ODN **20** was synthesized on a 0.2 μmol scale using the more commonly used protecting strategy (benzoyl for dA, isobutyryl for dG, and Ac for dC). The synthetic cycle with shortened capping time described earlier (Cycle 2) was used for the synthesis, and phosphoramidite **11** was employed for capping failure sequences. At the end of synthesis, DMTr group was removed, and cleavage was performed on the synthesizer with concentrated NH_4OH (900 min × 4) at rt. Deprotection was achieved by heating the concentrated NH_4OH solution in a screw capped vial at 65 ºC for 8 h. The ODN, which was labeled as **20**(c**11**c), was purified by catching failure sequences by polymerization as usual. RP HPLC analysis showed that the ODN was pure (see this ESI). The recovery yield of the purification process was estimated to be 76%. The purity is 93%. The results indicate that the catching failure sequences by polymerization purification method is equally effective when protecting groups that require harsher deprotecting conditions are used during ODN synthesis.

Capping with lower concentration polymerizable phosphoramidite solution

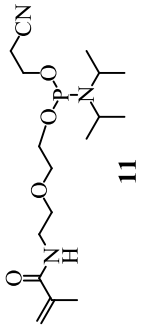
In our preliminary communication, we used a capping solution with a concentration of 0.2 M to cap failure sequences (S. Fang et al, *Chem. Commun.*, 2011, **47**, 1345). In the present work, we reduced the concentration of capping solution to 0.15 M. Good purification results were obtained in both cases. In the coupling steps, the concentration of nucleoside phosphoramidite monomers we used was 0.1 M. We were interested in further reducing the concentration of the capping solution to this value. For the purpose, ODN **20** was synthesized using the procedure with shortened capping time (Cycle 2). Capping failure sequences was performed with a 0.1 M solution of **10**. The resulting ODN, which is labeled as **20**(c**10**lc), was purified by catching failure sequences by polymerization as usual. RP HPLC analysis showed that the ODN was equally pure (see this ESI). The recovery yield of ODN was estimated to be 90%. The purity is 99%.

Purification by polymerization in air

So far in all of our studies, the polymerization step, in which failure sequences were incorporated into a polyacrylamide gel, was performed under a nitrogen atmosphere to minimize the chances of termination of polymerization by oxygen. This may not be convenient for some applications such as high throughput purification. Noting that excess initiators were used and cross-linking could connect terminated polymer segments, we tested to perform the step in air in a 1.5 mL centrifuge tube. A portion of ODN **20**(c**11**s) in a centrifuge tube was simply dissolved in water and polymerized by adding polymerization solution and initiators to the tube directly. Interestingly, the polymerization speed was not found slower than that under nitrogen atmosphere, and the gel was formed within 5 min. After waiting for complete polymerization for 1 h, extraction of full-length sequences with water was also carried out directly in the same tube.

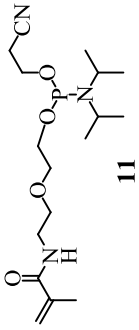
The remaining steps were the same as in the general purification procedure. RP HPLC showed that the ODN was equally pure (see this ESI). The recovery yield was estimated to be 87%. The purity is 100%. Polymerization under nitrogen in large scale purifications should be quite convenient, and is not expected to add any significant cost to the process. However, for high throughput purification, handling many samples at the same time under a nitrogen atmosphere may require special equipment. Therefore, the results are important for applying the technology in high throughput ODN purification.

^{1}H NMR of compound **11** in $CDCl_3$ at 400 MHz



11

^{13}C NMR of compound **11** in $CDCl_3$ at 100 MHz



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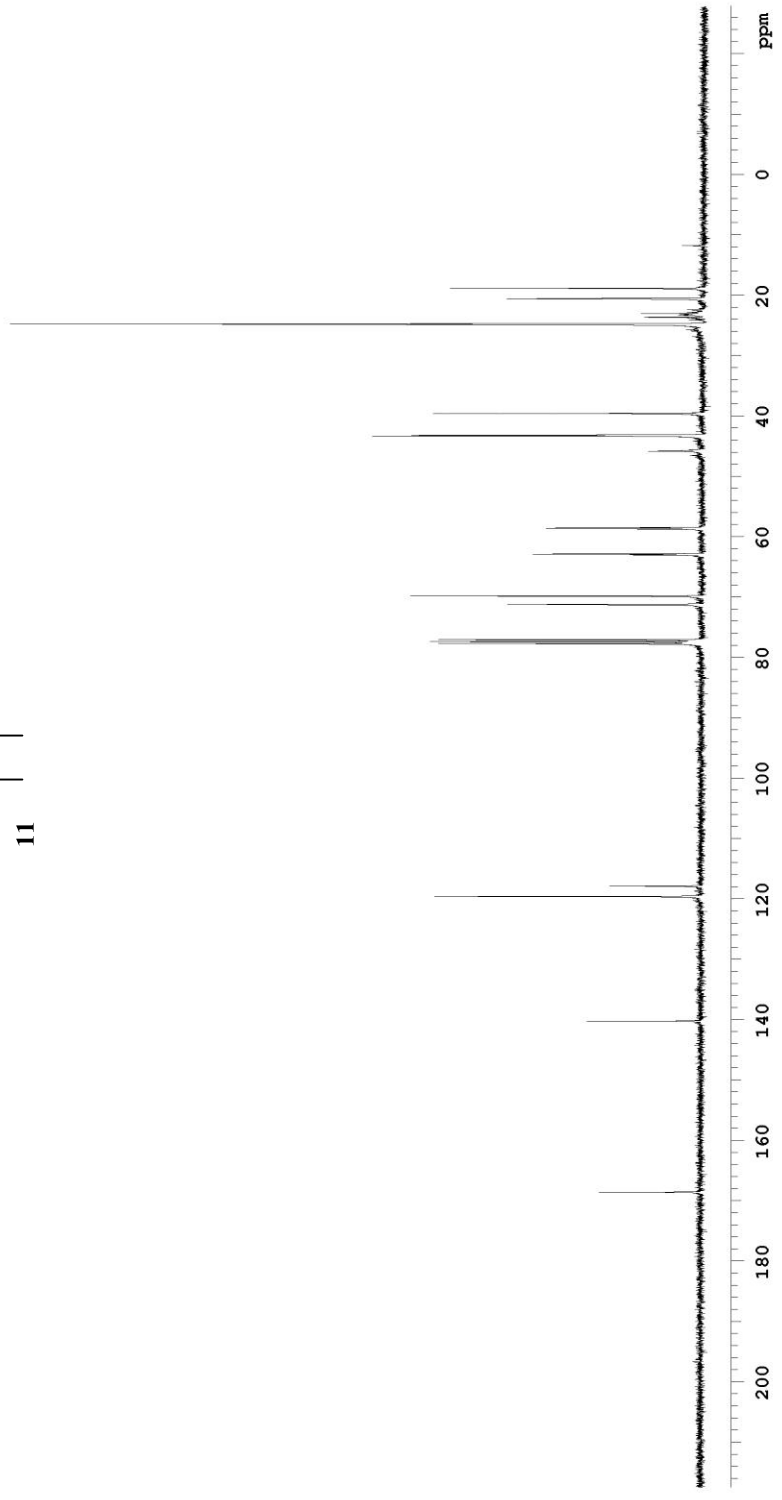

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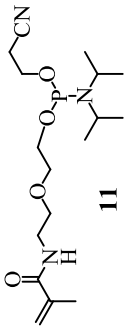

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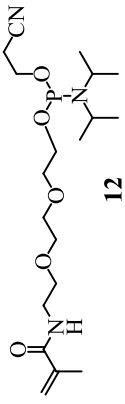

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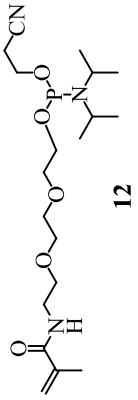

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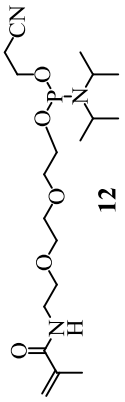
^{31}P NMR of compound **12** in $CDCl_3$ at 162 MHz



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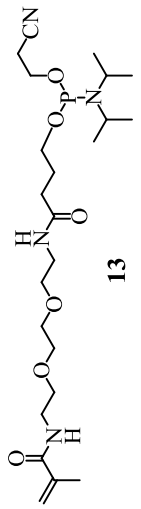

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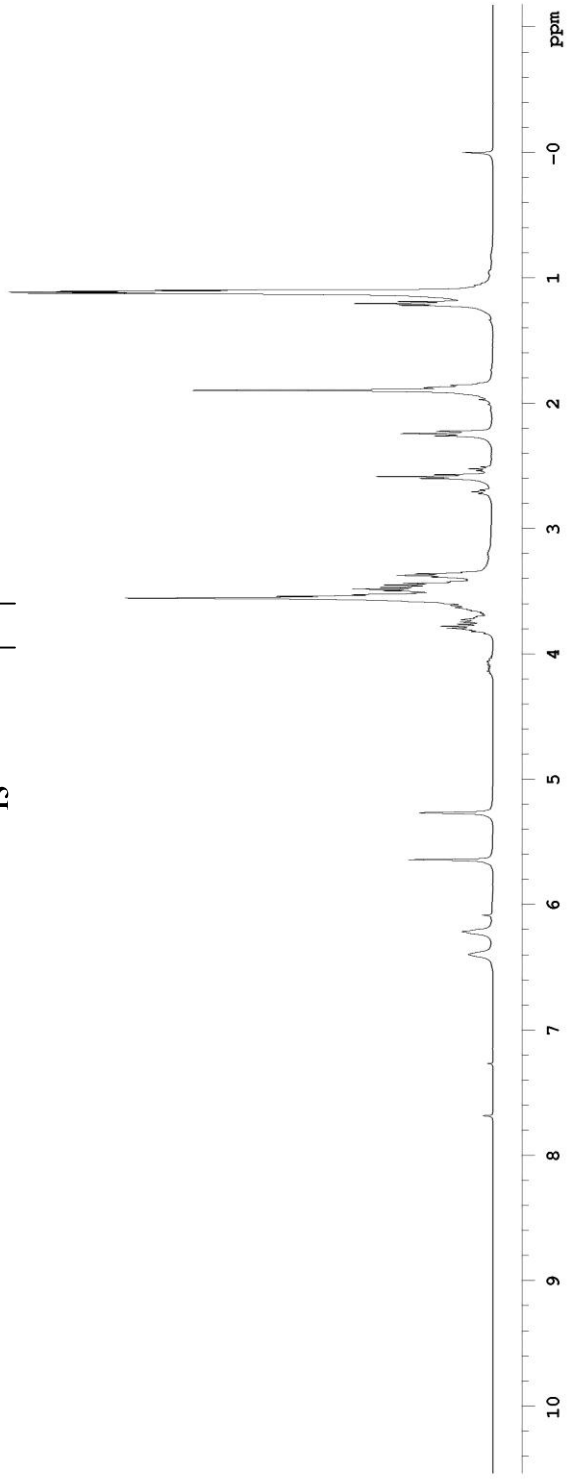

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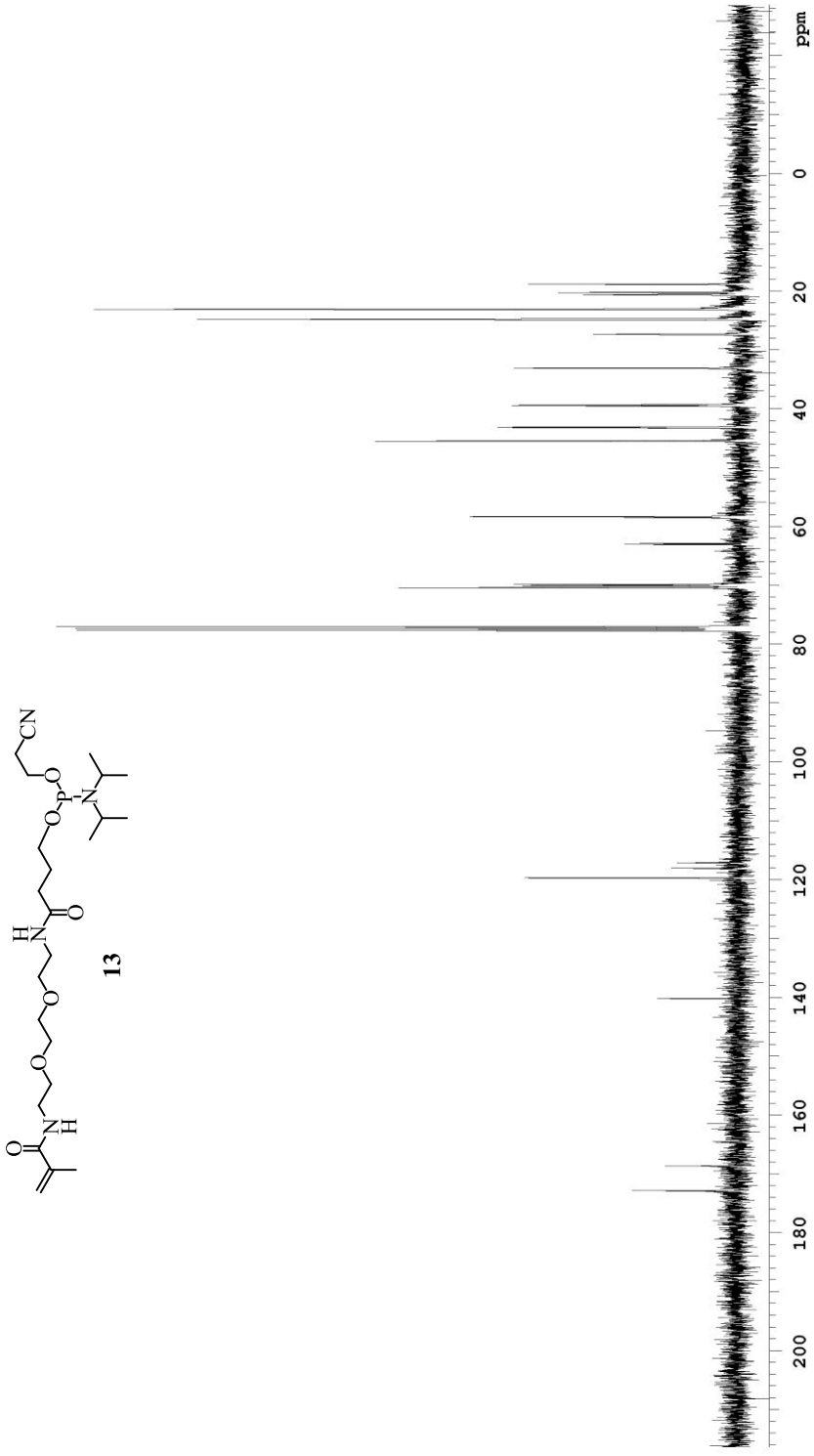

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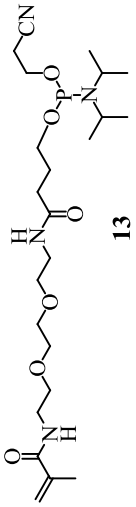

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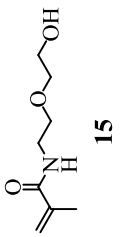
^{1}H NMR of compound **15** in $CDCl_3$ at 400 MHz

15

^{13}C NMR of compound **15** in $CDCl_3$ at 100 MHz



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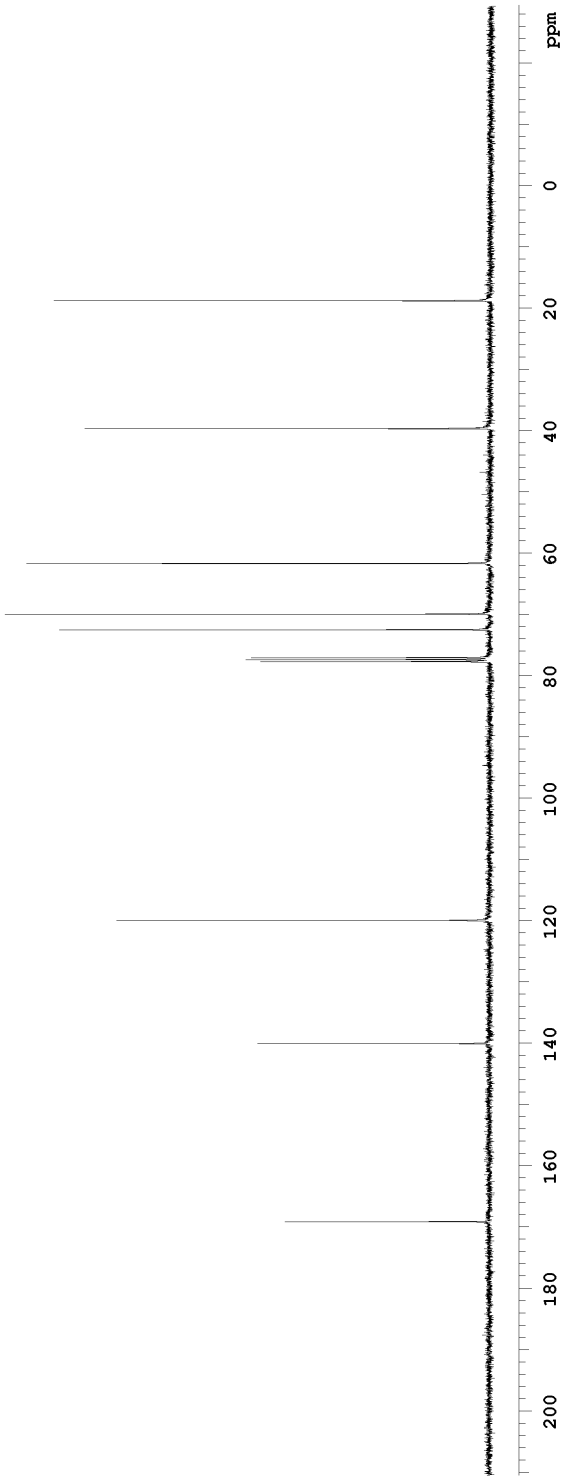

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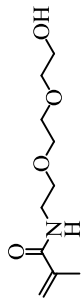

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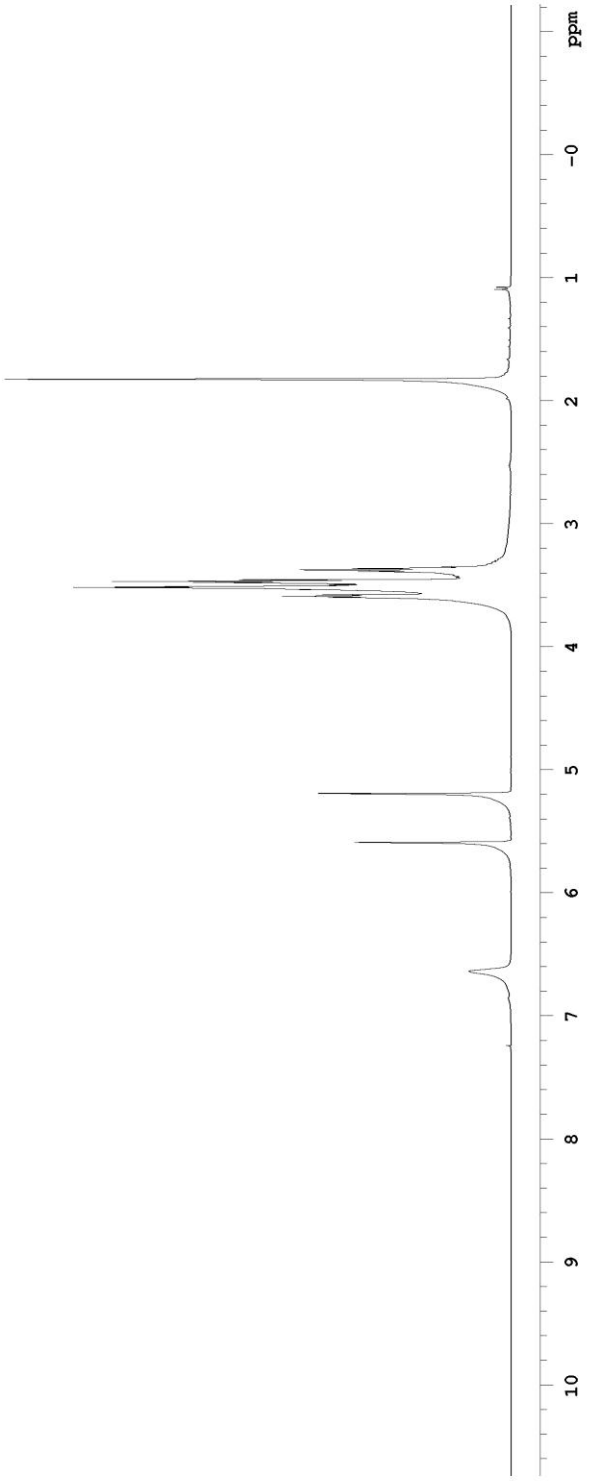
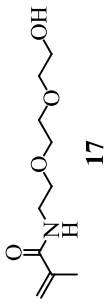


^{13}C NMR of compound **17** in $CDCl_3$ at 100 MHz



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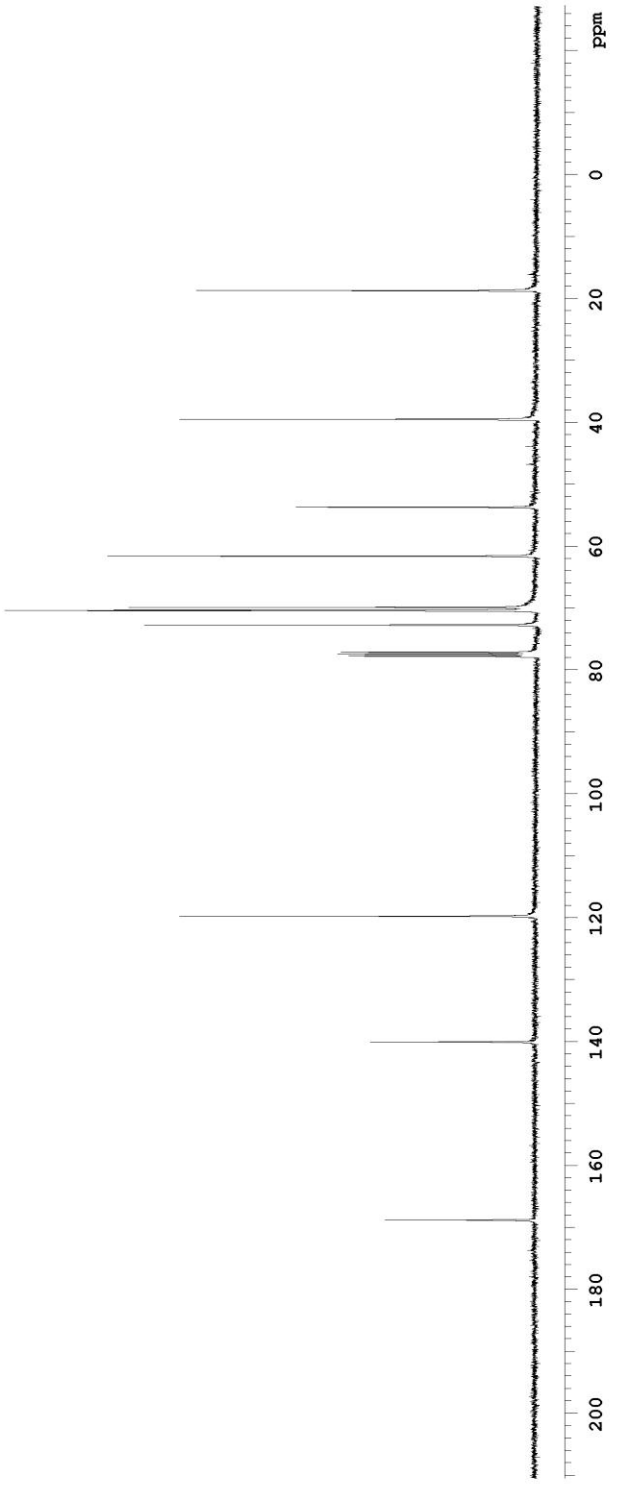
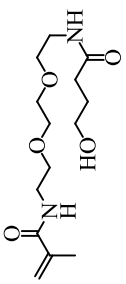


^{1}H NMR of compound **19** in $CDCl_3$ at 400 MHz



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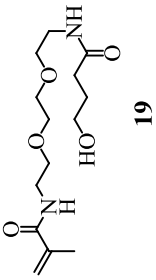
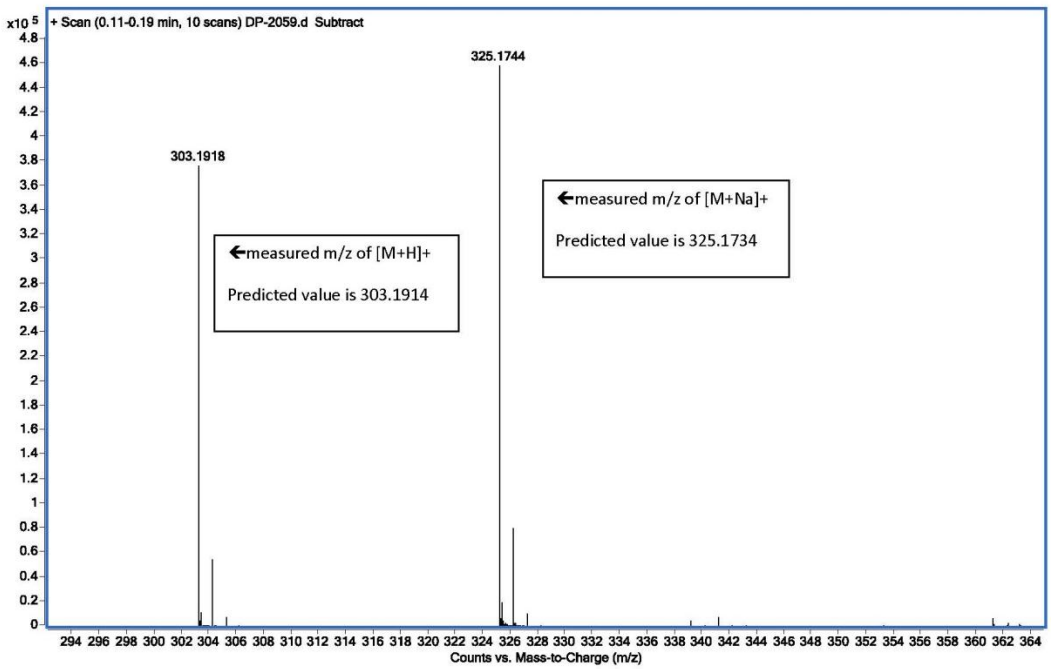
^{13}C NMR of compound **19** in $CDCl_3$ at 100 MHz

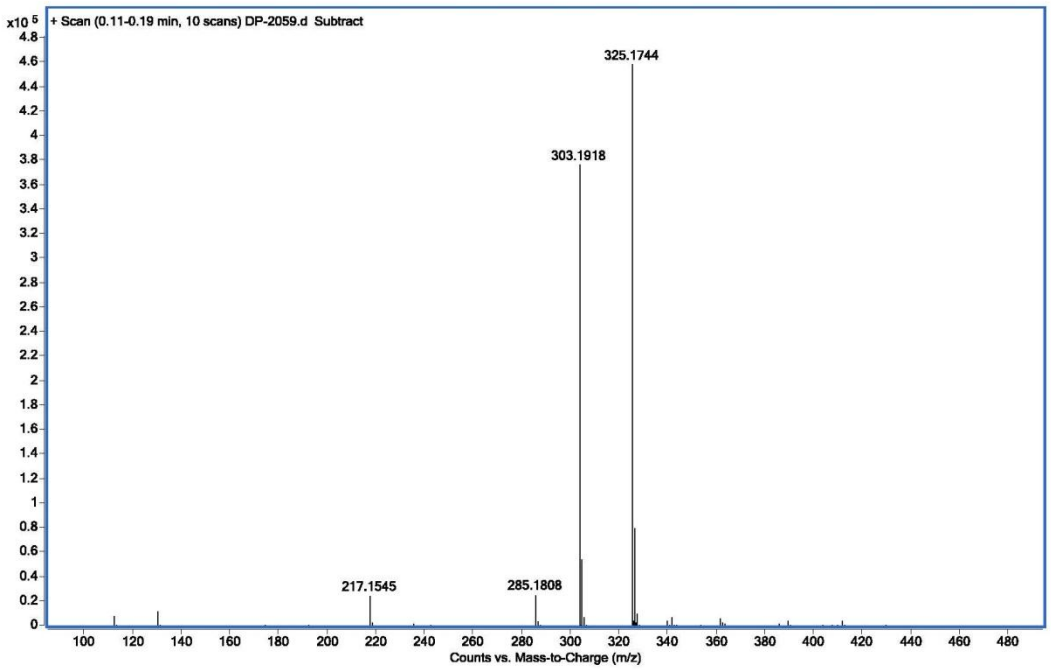


19

Mass spectra of compound **19**

Durga Pokharel DP-2059 ESI+

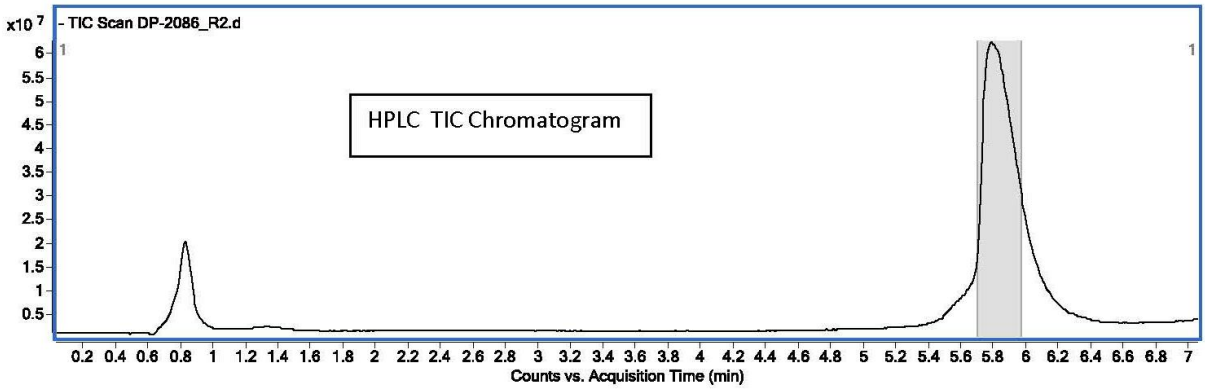




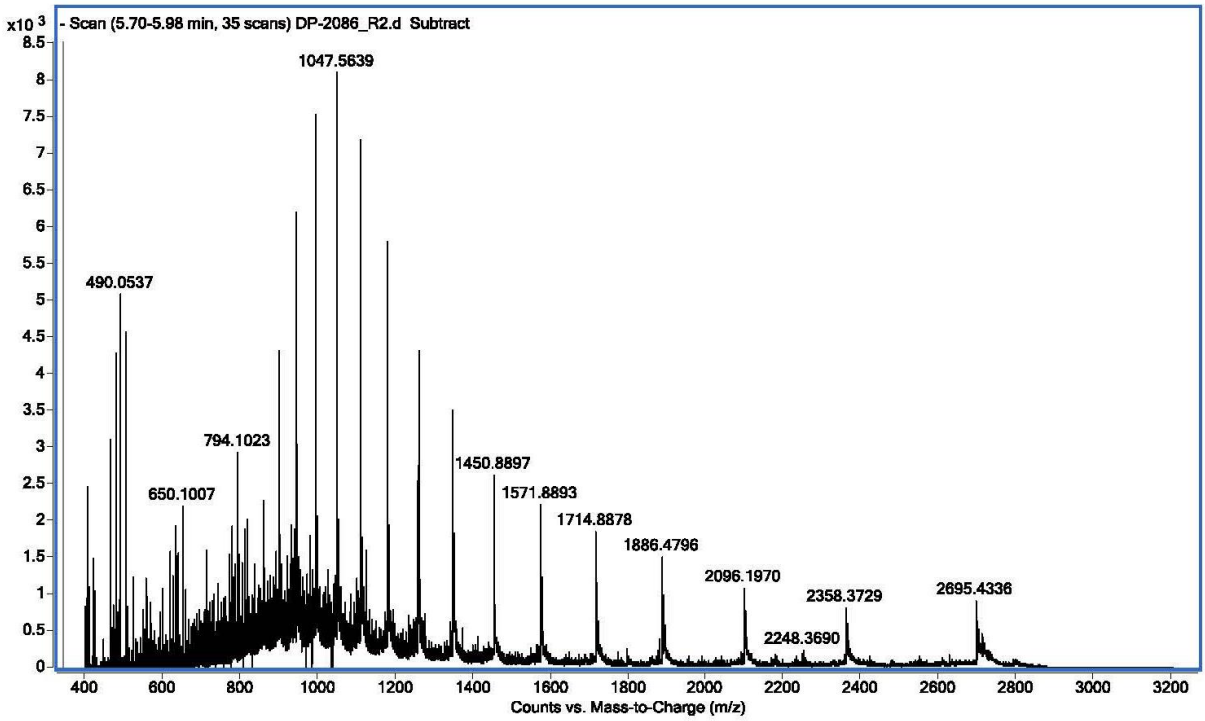
LC MS of ODN **23**

Sample 2086

Negative ion ESI



Raw data ESI- spectrum of highlighted area of the chromatogram:



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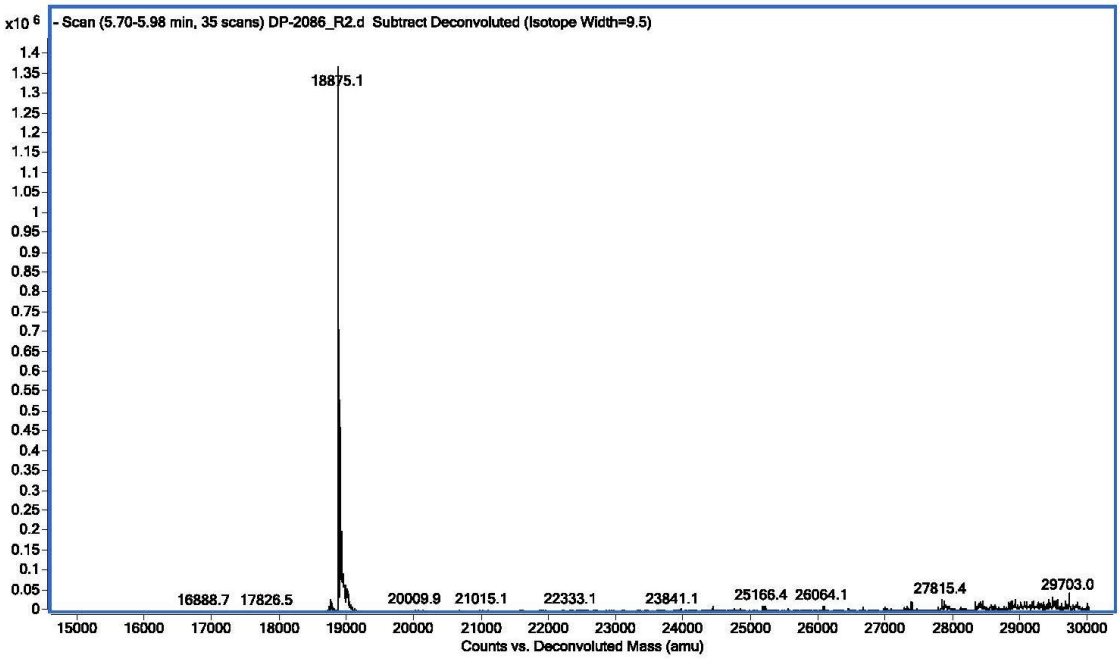
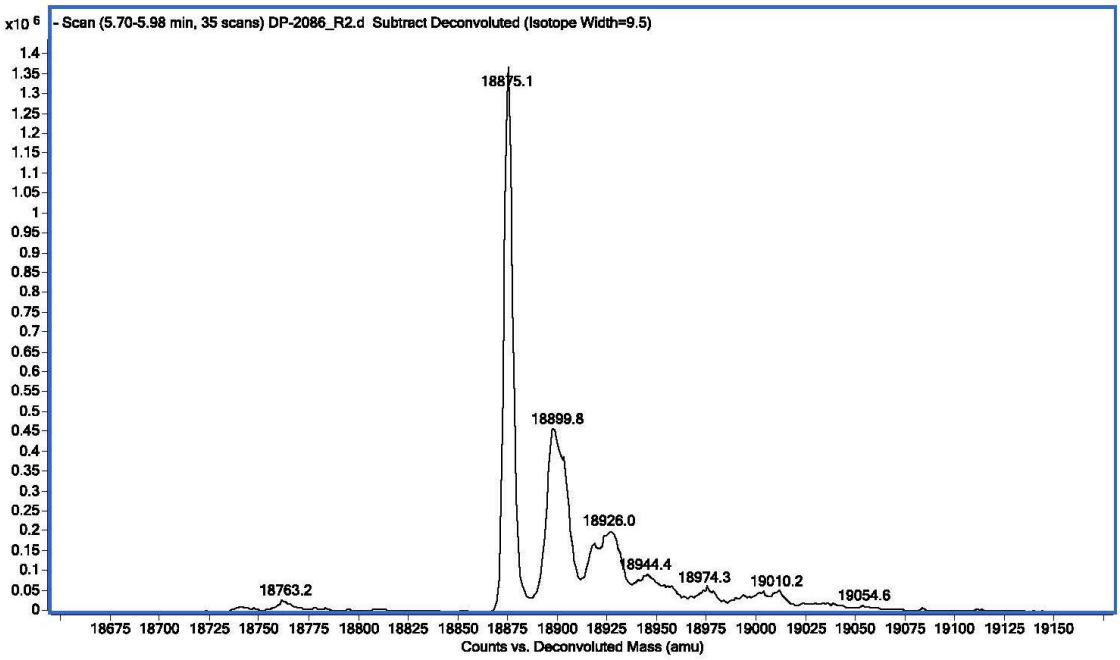
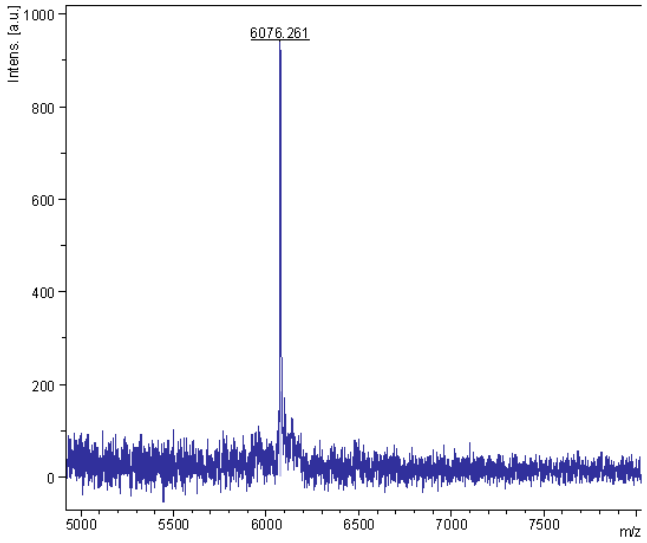




MALDI-TOF MS of ODN **24**

8-Oxo-G-20merSequence - 8-Oxo-G-20merSequence

8-Oxo-G-20merSequence



m/z	S/N	Quality Fac.	Res.	Intens.	Area
6076.261	13.3		1300	940.00	5412

Target

Position	F5

Laser

Laser beam attenuation	52.222
Laser repetition rate	60 Hz
Number of shots	1200

Spectrometer

positive voltage polarity	POS
PIE delay	50 ns
Ion source voltage 1	20 kV
Ion source voltage 2	18.4 kV
Lens voltage	7 kV
Linear detector voltage	2.64 kV
Deflection on	true
Deflection mass	2000 Da
SampleRate	1 ns
Reflector voltage 1	0 kV
Reflector detector voltage	0 kV
MSMS parent mass	

Instrument

Instrument type	microflex
Serial instrument number	256969.00064
Name of computer	FLEX-PC
Operator ID or name	durga
flexControl version	flexControl 3.3.108.0
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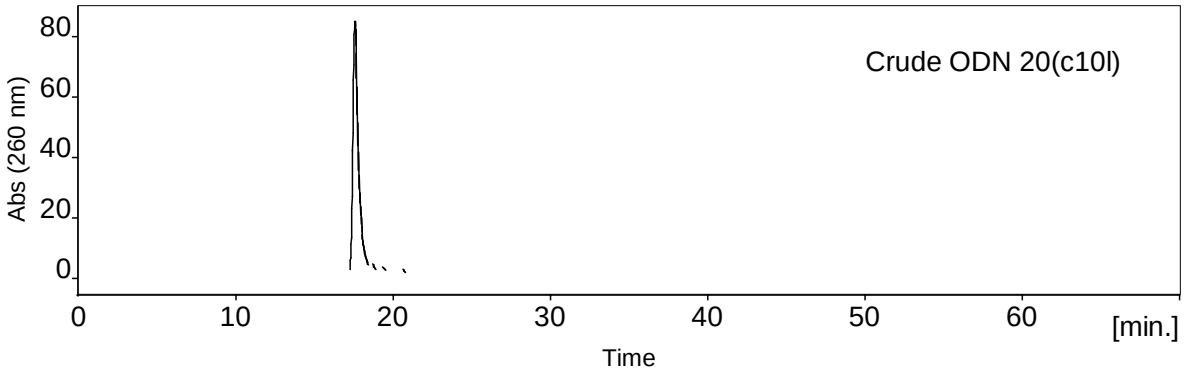
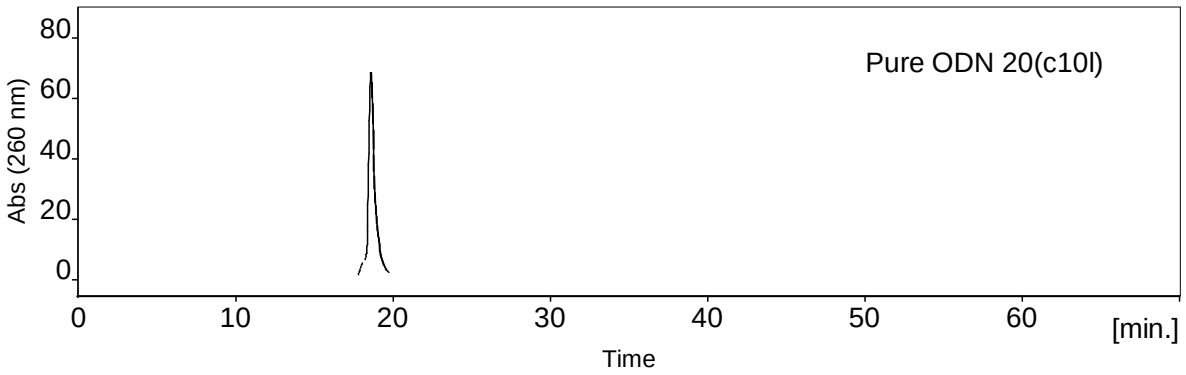
RP HPLC profile of crude ODN **20**(c**10**l)



RP HPLC profile of ODN **20**(c**10**l) purified by catching by polymerization



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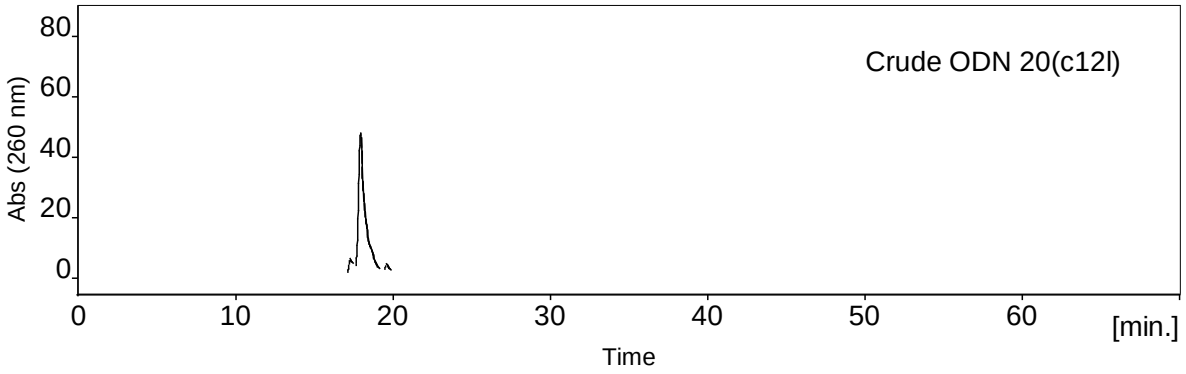

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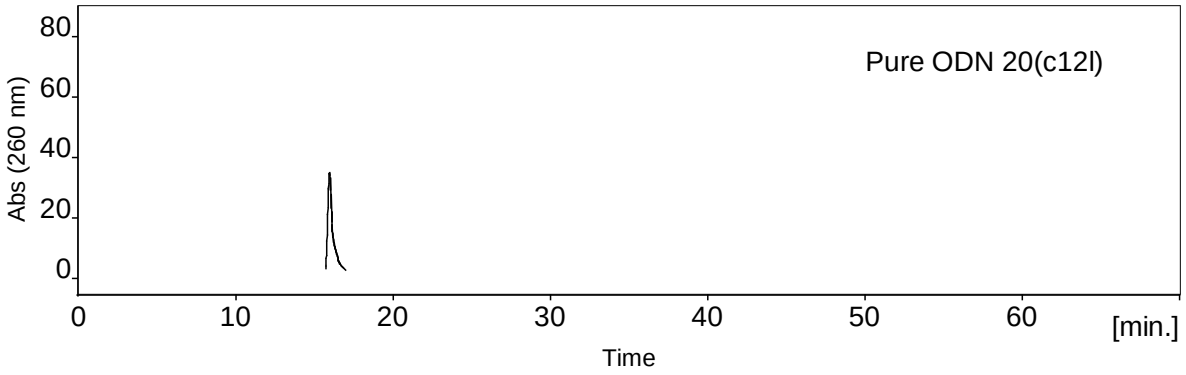

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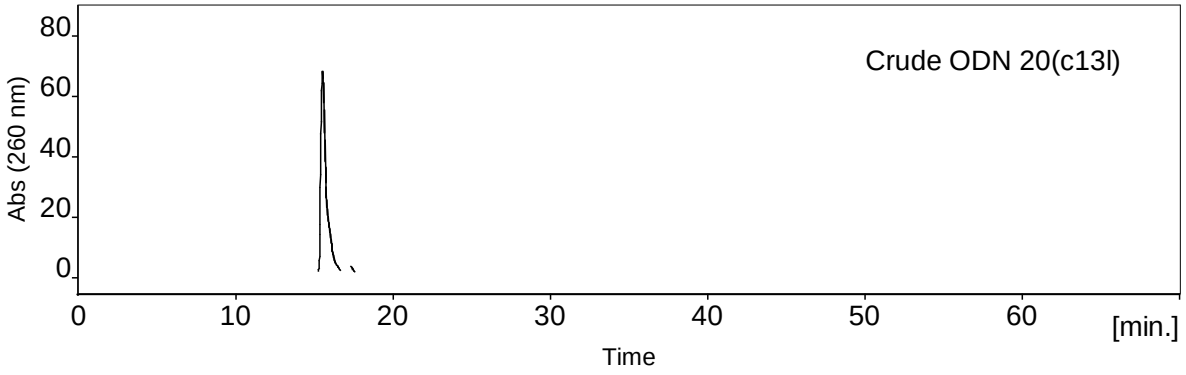

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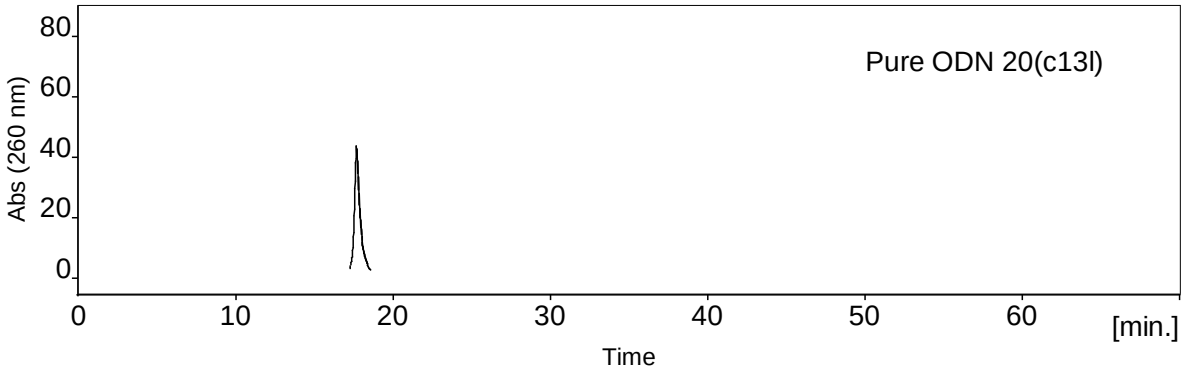

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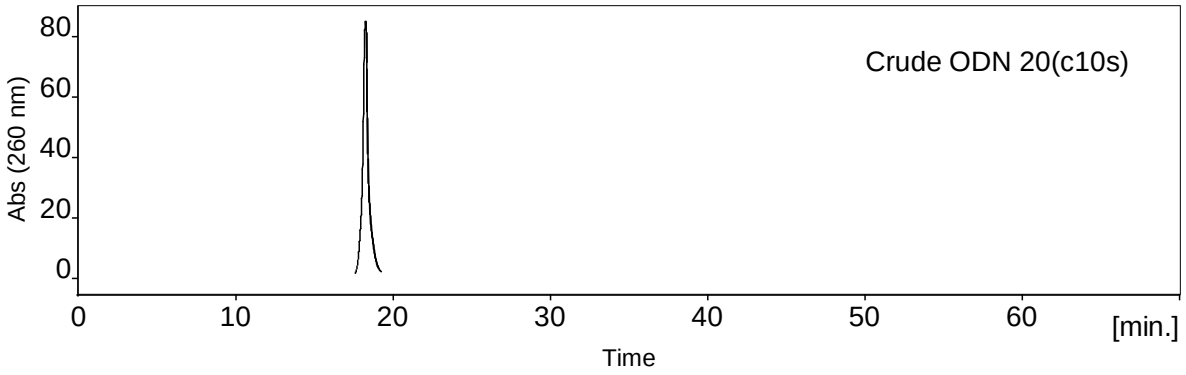

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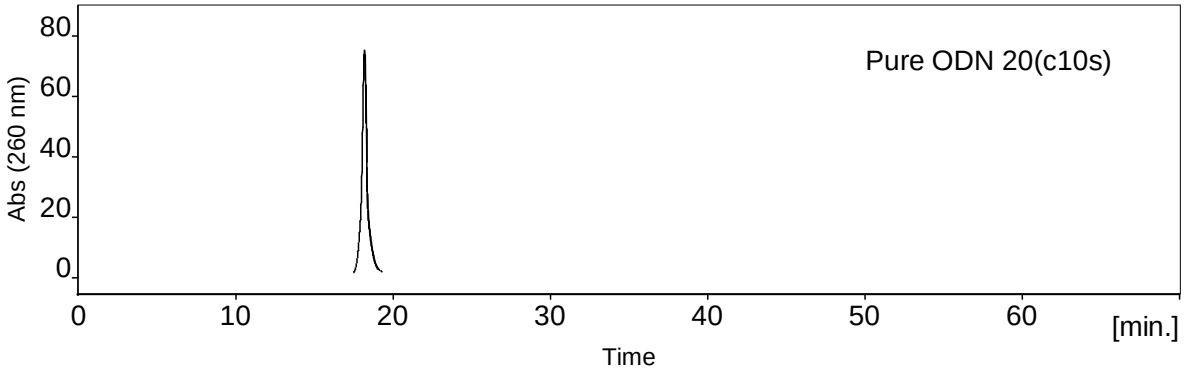

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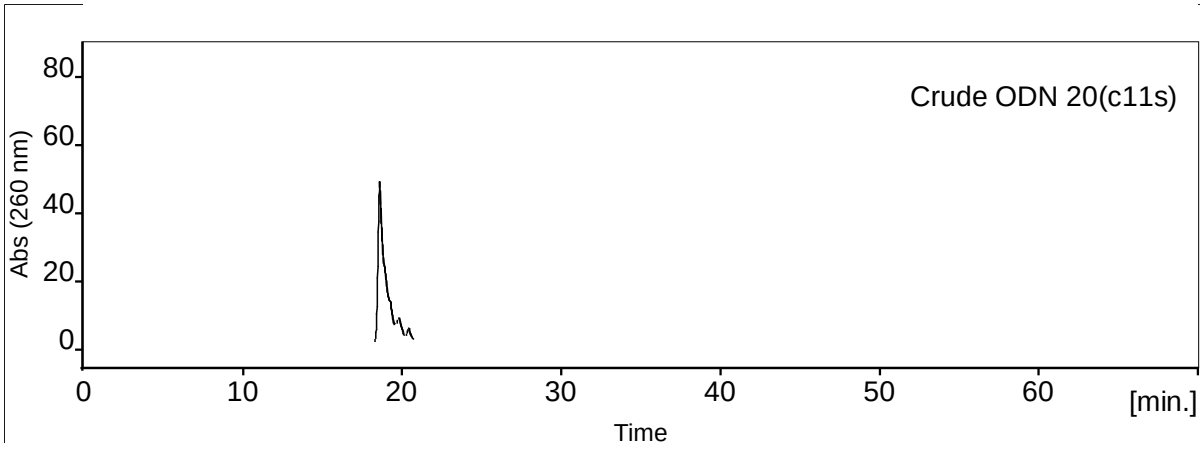
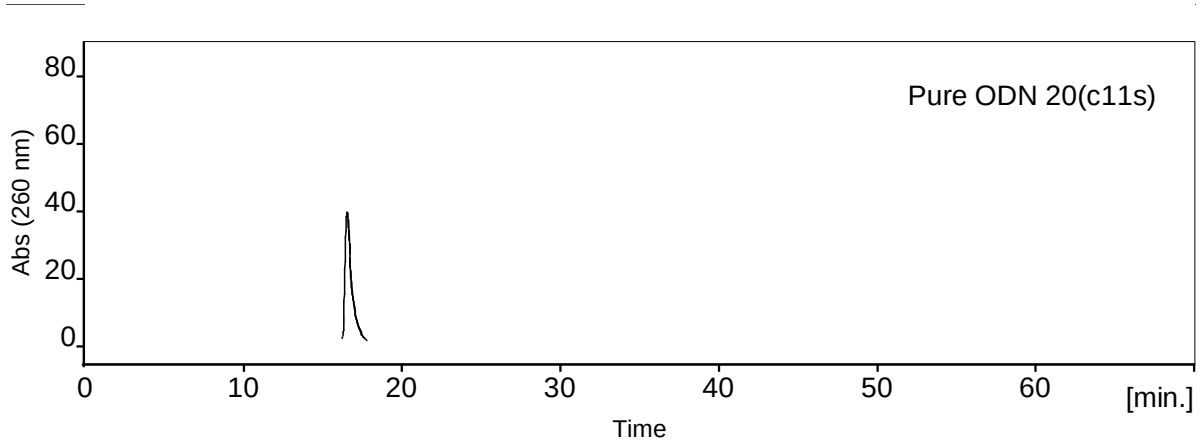


RP HPLC profile of ODN **20**(c**11**s) purified by catching by polymerization



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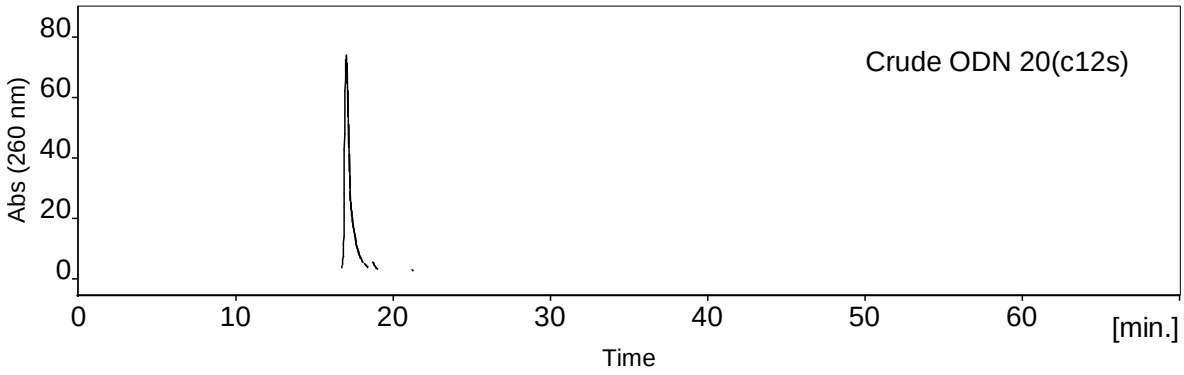
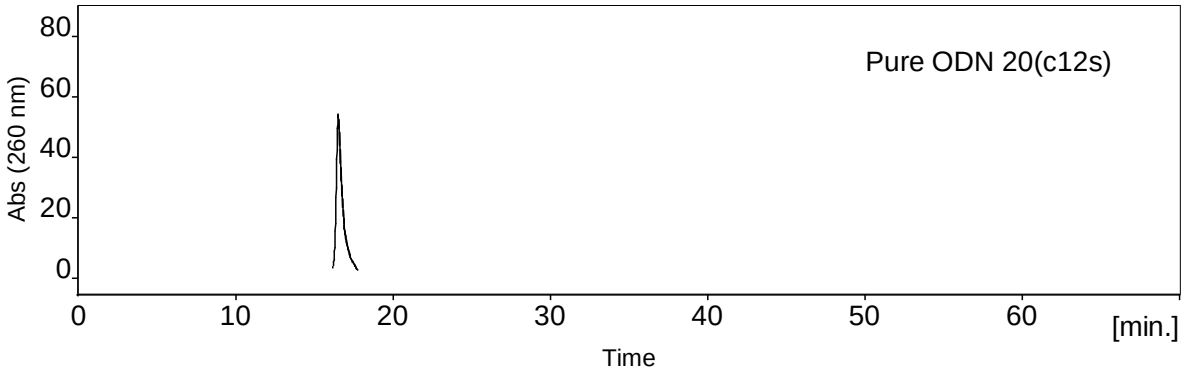
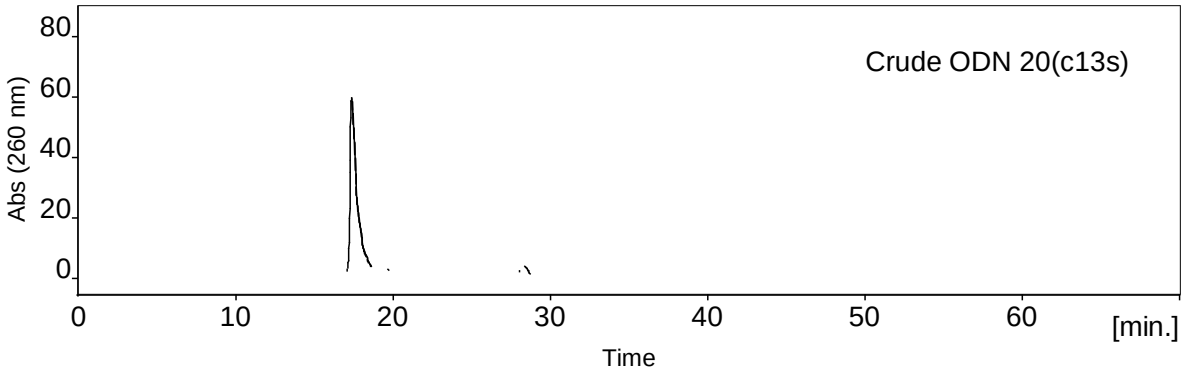


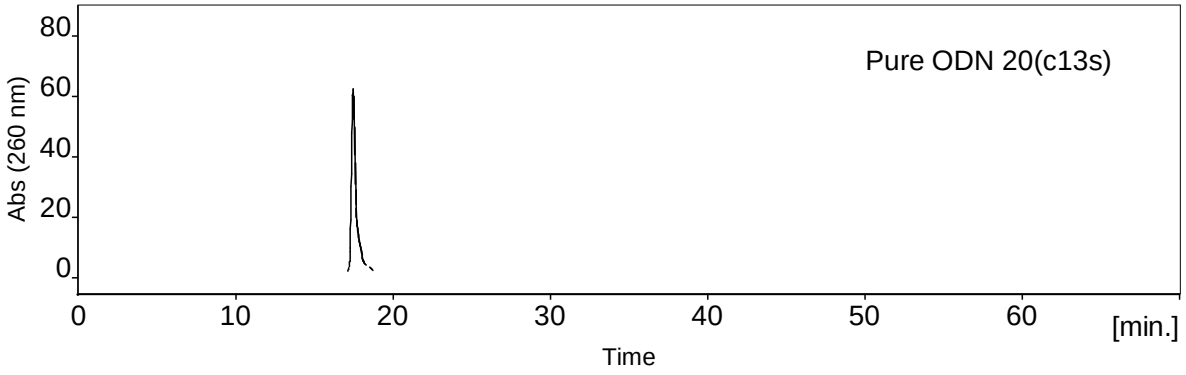
RP HPLC profile of ODN **20**(c**12**s) purified by catching by polymerization



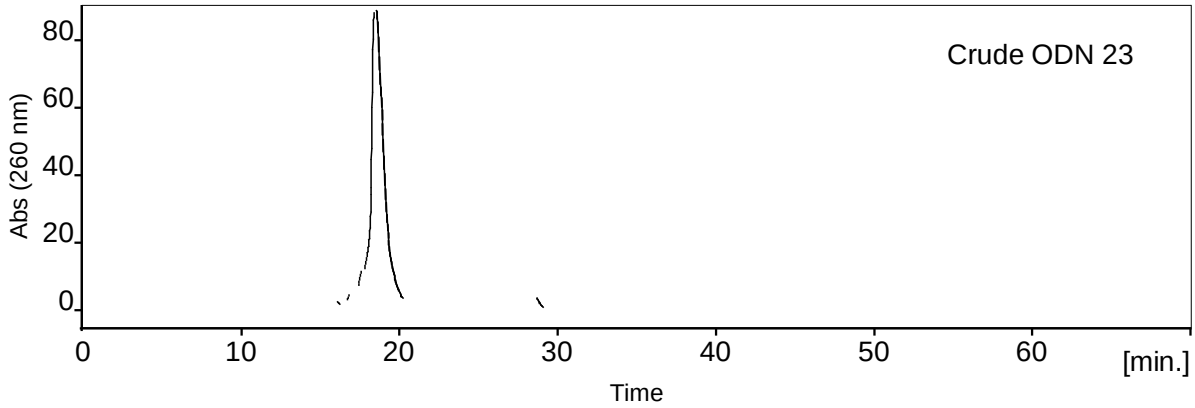
RP HPLC profile of crude ODN **20**(c**13**s)



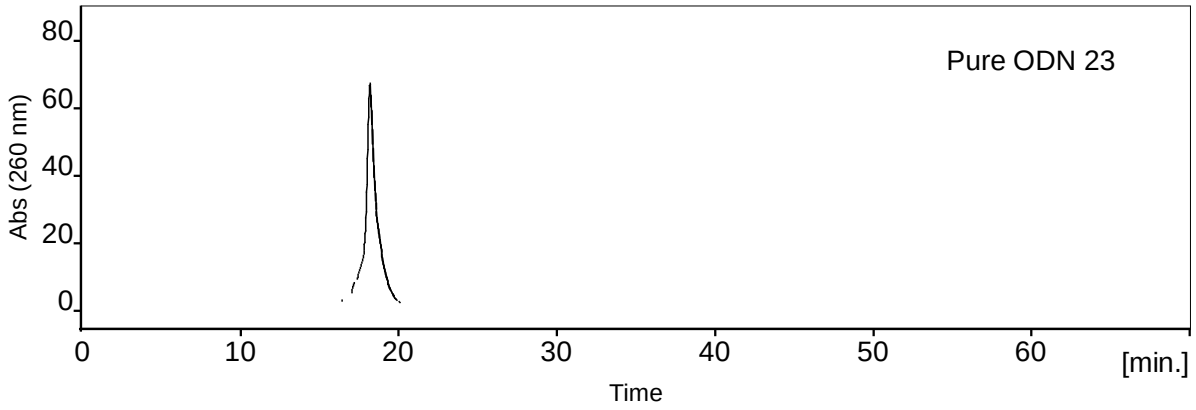
RP HPLC profile of ODN **20**(c**13**s) purified by catching by polymerization



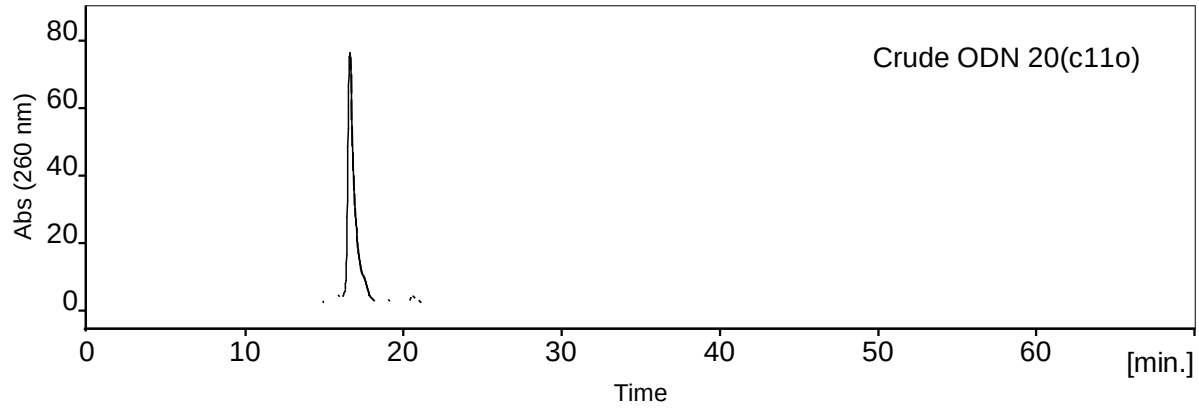
RP HPLC profile of crude ODN **23**



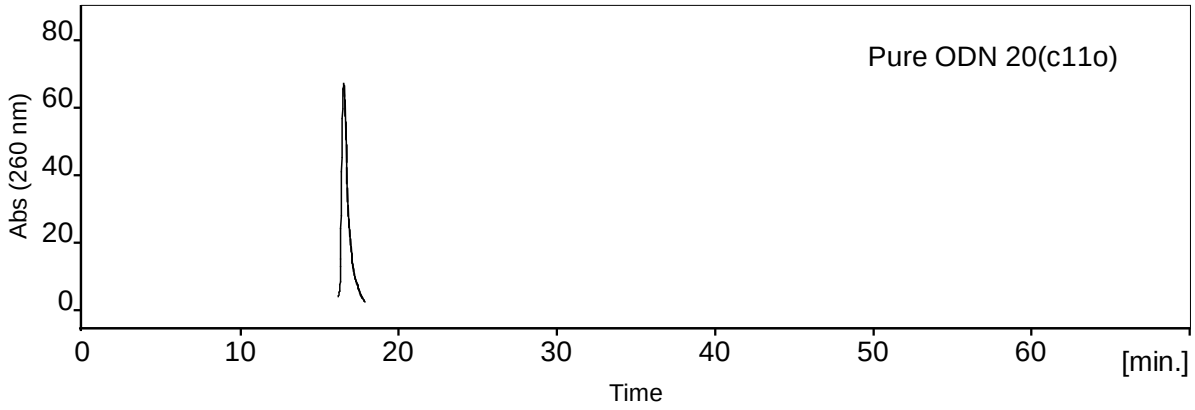
RP HPLC profile of ODN **23** purified by catching by polymerization



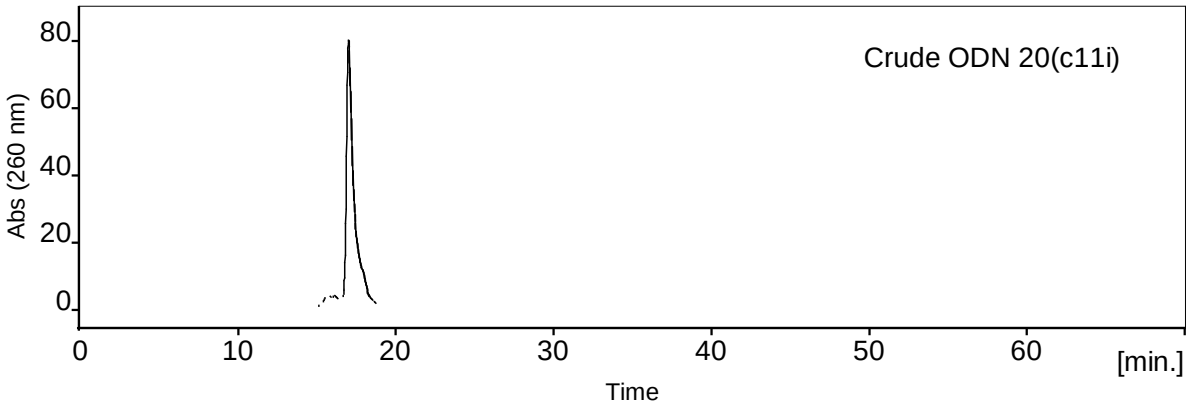
RP HPLC profile of crude ODN **20**(c**11**o)



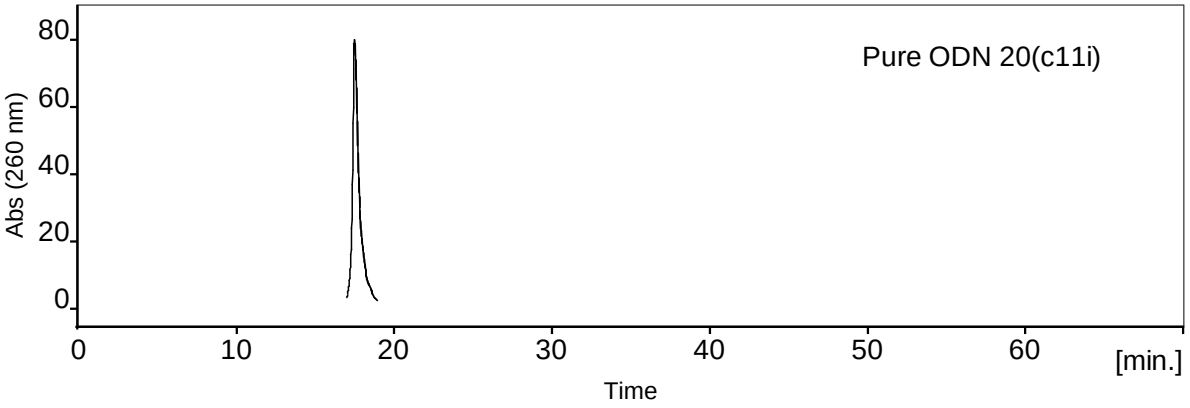
RP HPLC profile of ODN **20**(c**11**o) purified by catching by polymerization



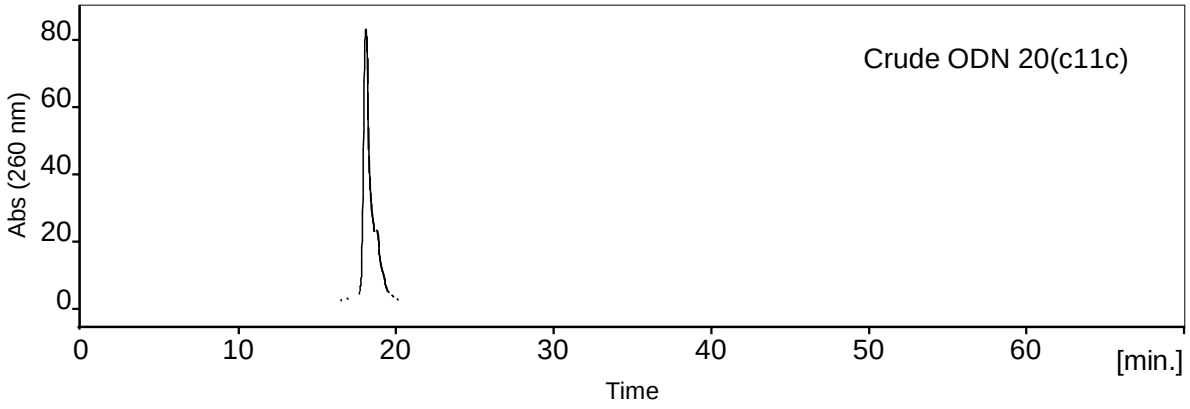
RP HPLC profile of crude ODN **20**(c**11**i)



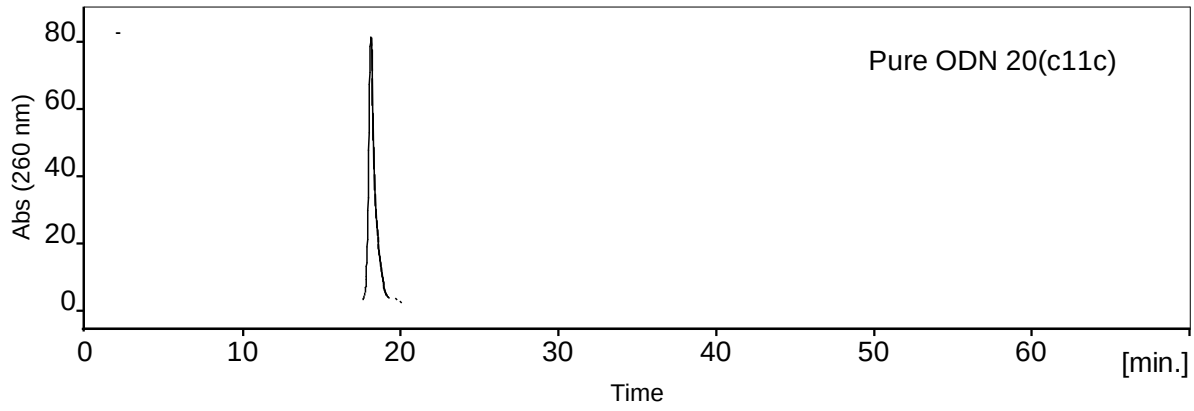
RP HPLC profile of ODN **20**(c**11**i) purified by catching by polymerization



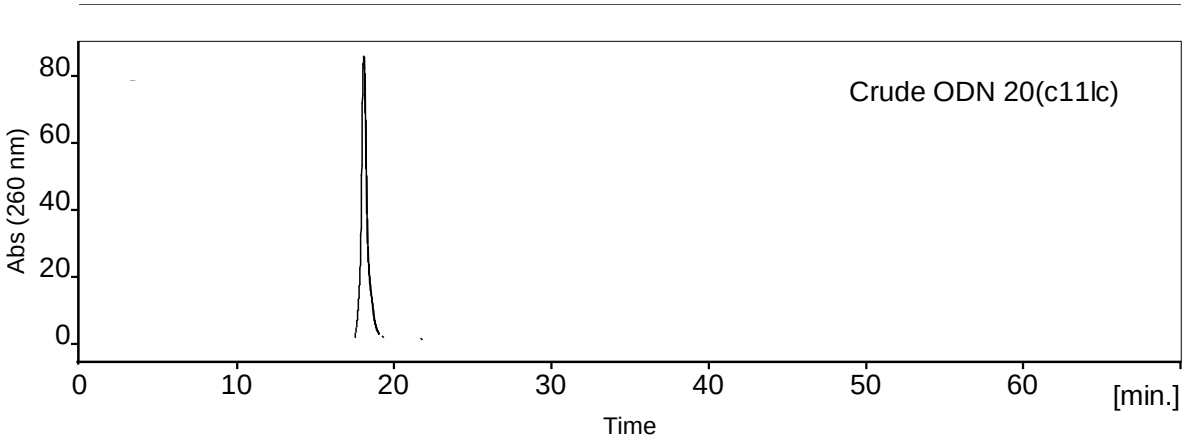
RP HPLC profile of crude ODN **20**(c**11**c)



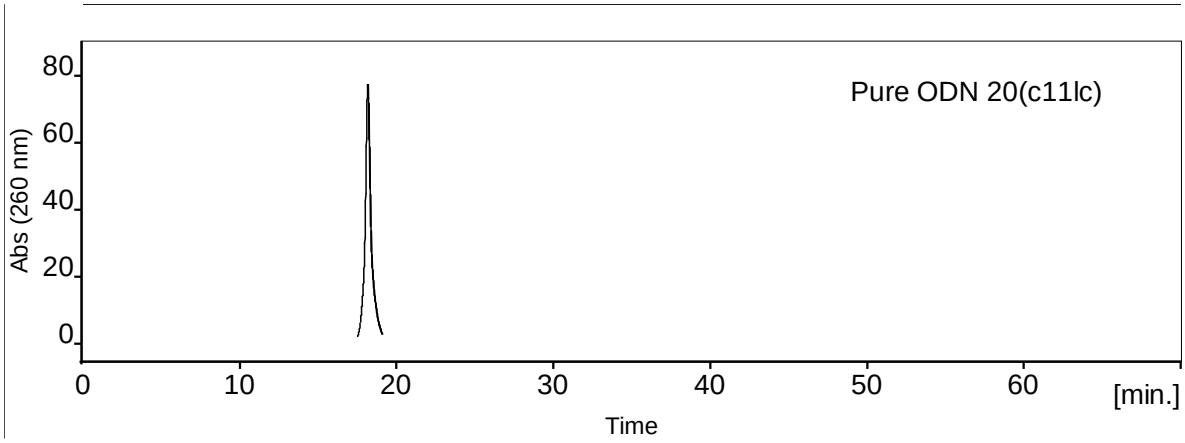
RP HPLC profile of ODN **20**(c**11**c) purified by catching by polymerization



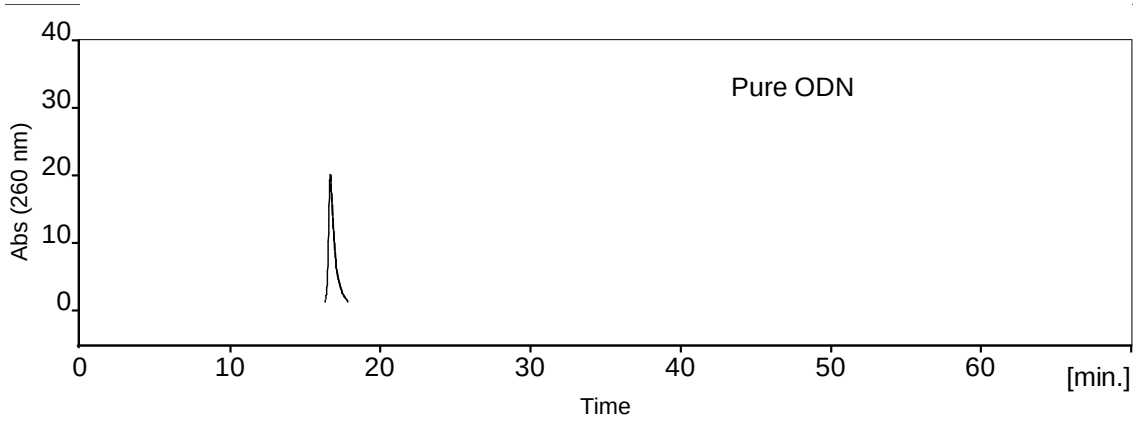
RP HPLC profile of crude ODN **20**(c**11**lc)



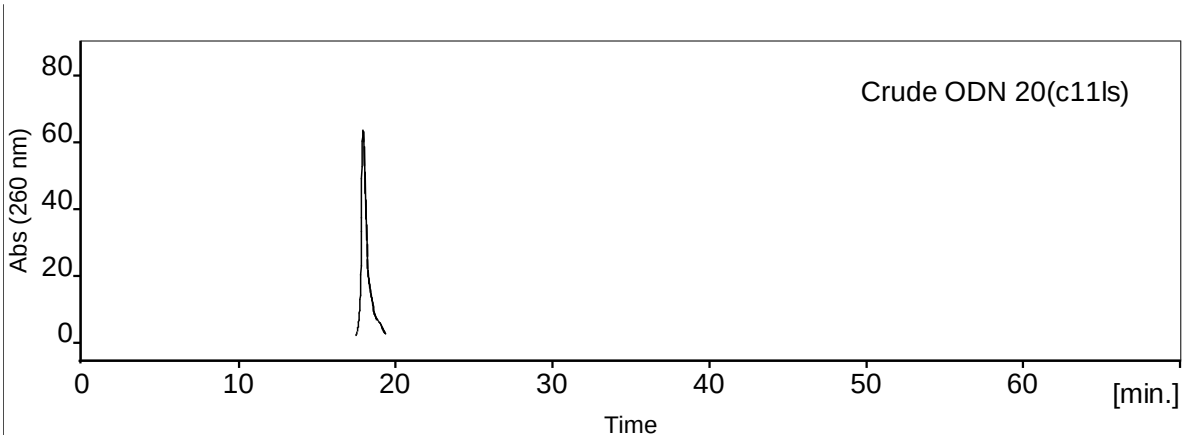
RP HPLC profile of ODN **20**(c**11**lc) purified by catching by polymerization



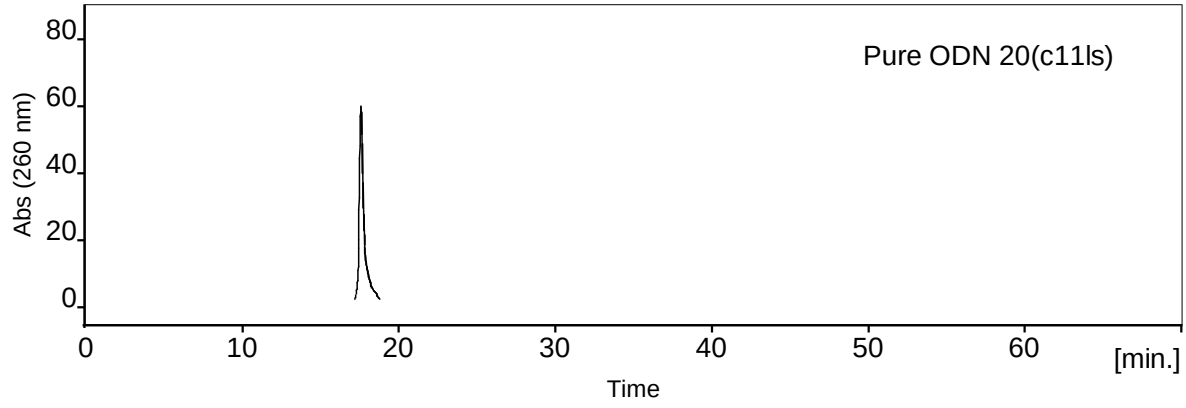
RP HPLC profile of ODN **20** purified by catching by polymerization; the polymerization step was carried in air in a centrifuge tube



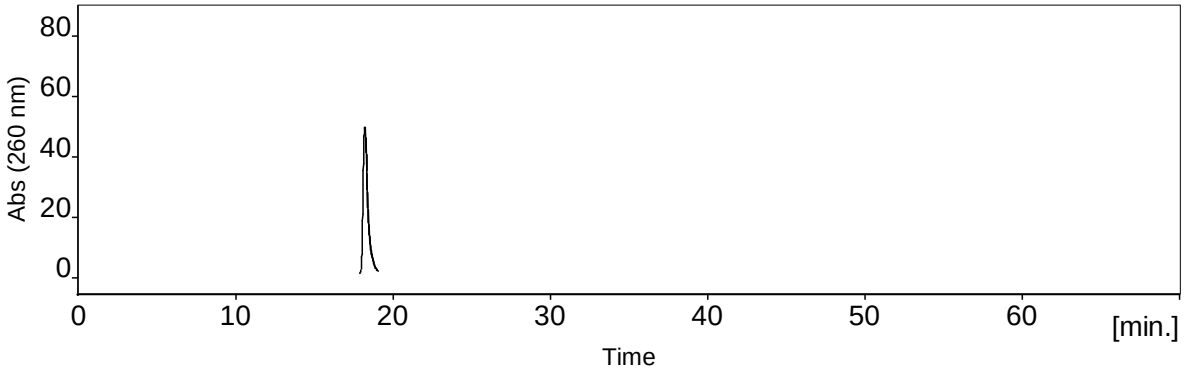
RP HPLC profile of crude ODN **20**(c**11**ls)



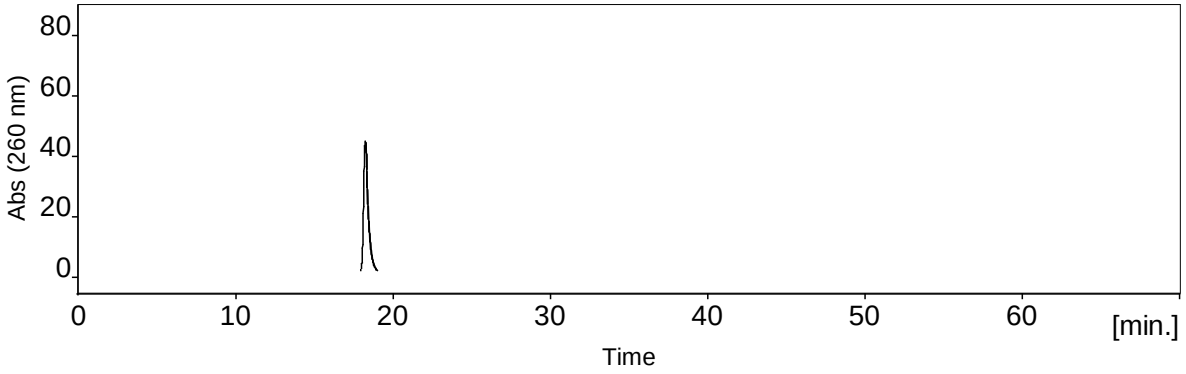
RP HPLC profile of ODN **20**(c**11**ls) purified by catching by polymerization



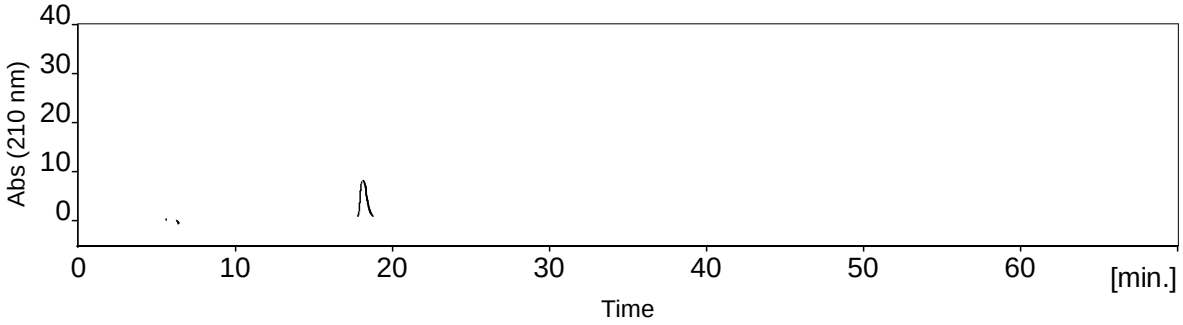
RP HPLC profile of crude ODN **20**(c**11**dci)



RP HPLC profile of ODN **20**(c**11**dci) purified at 3 µmol scale using the catching failure sequences by polymerization method

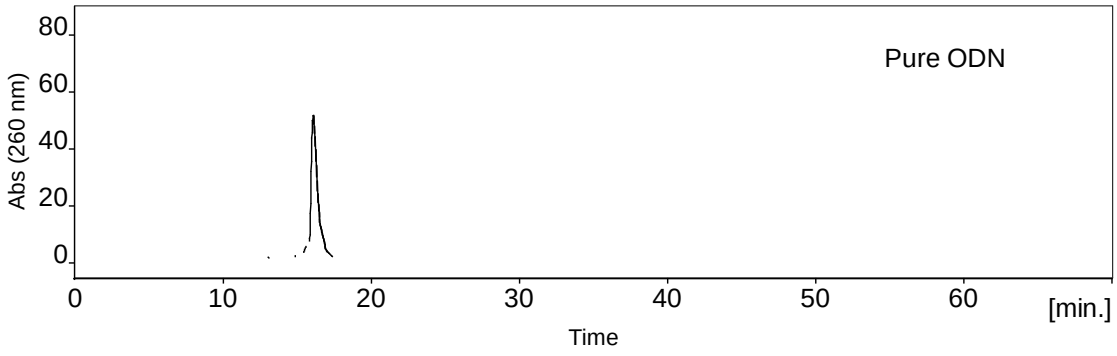


RP HPLC profile of ODN **20**(c**11**dci) purified at 3 µmol scale using the catching failure sequences by polymerization method; the profile was generated by UV detection at 210 nm

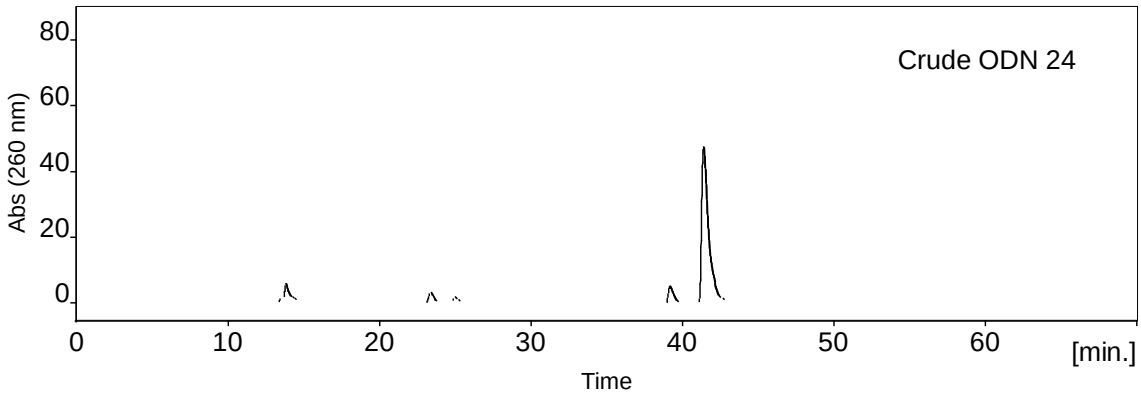


Ion-exchange HPLC analysis of ODN **20**

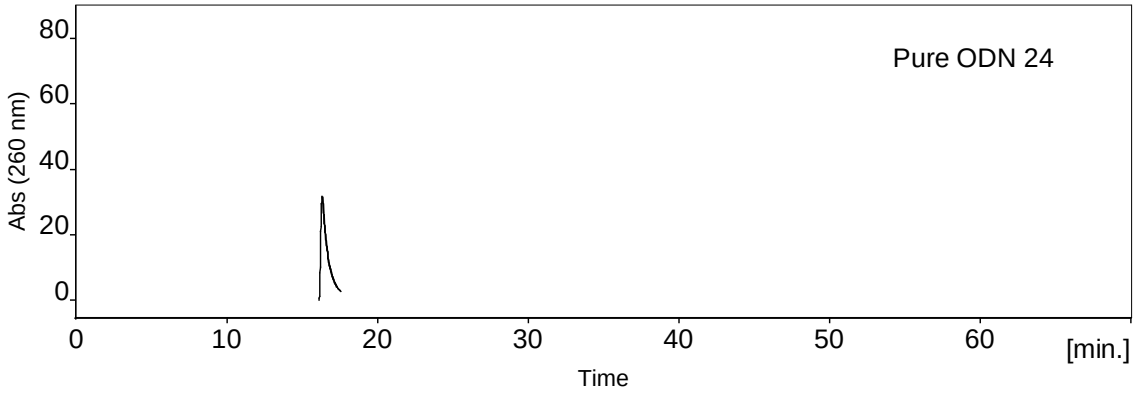
The conditions described in the general methods section were used. For analysis, 20 μL solution from the remaining ODN **20** (c**12**s) solution in the purification by catching failure sequences by polymerization experiment following the general ODN purification procedure was injected into HPLC.



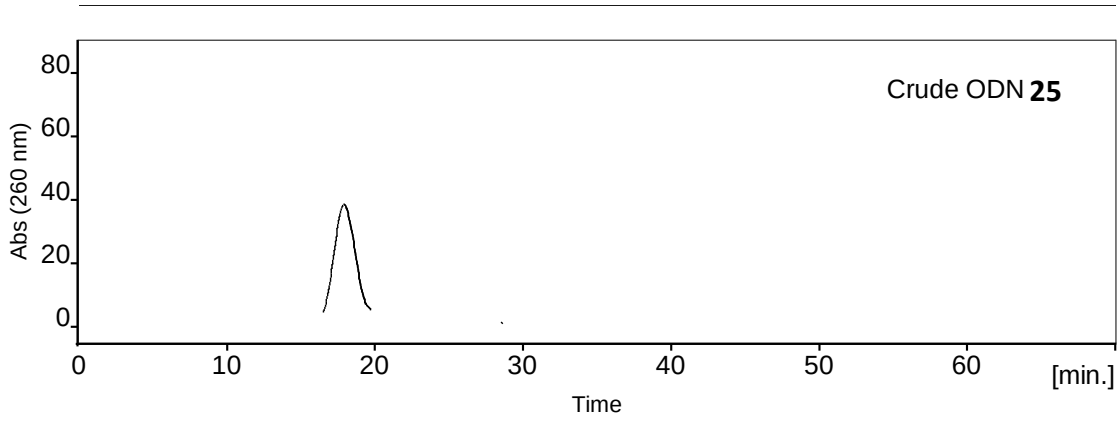
RP HPLC profile of crude ODN **24**



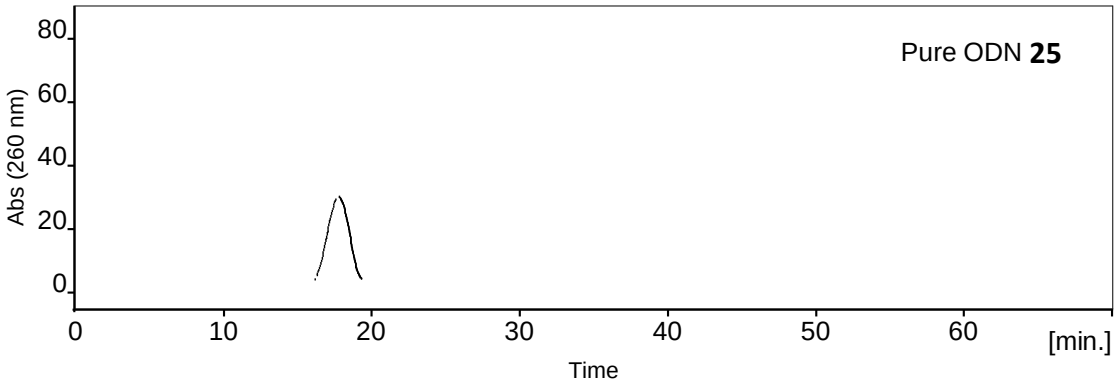
RP HPLC profile of pure ODN **24**



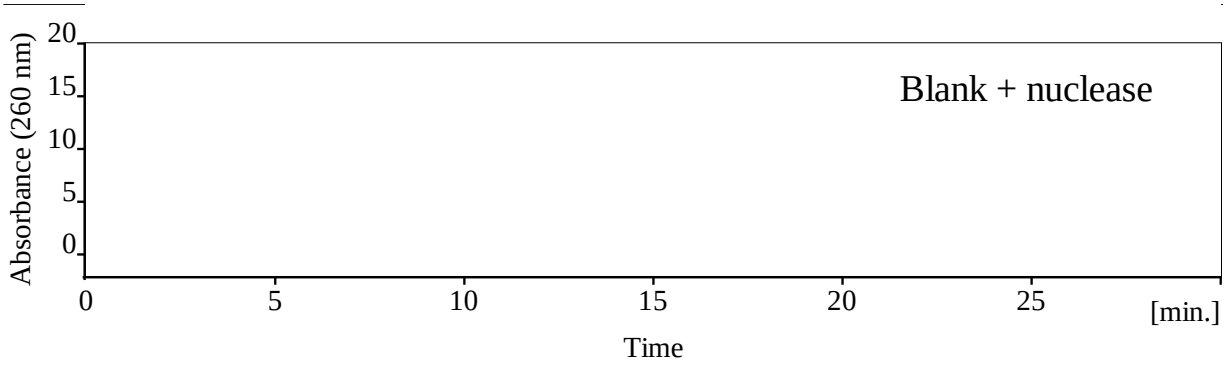
RP HPLC profile of crude ODN mixture **25**



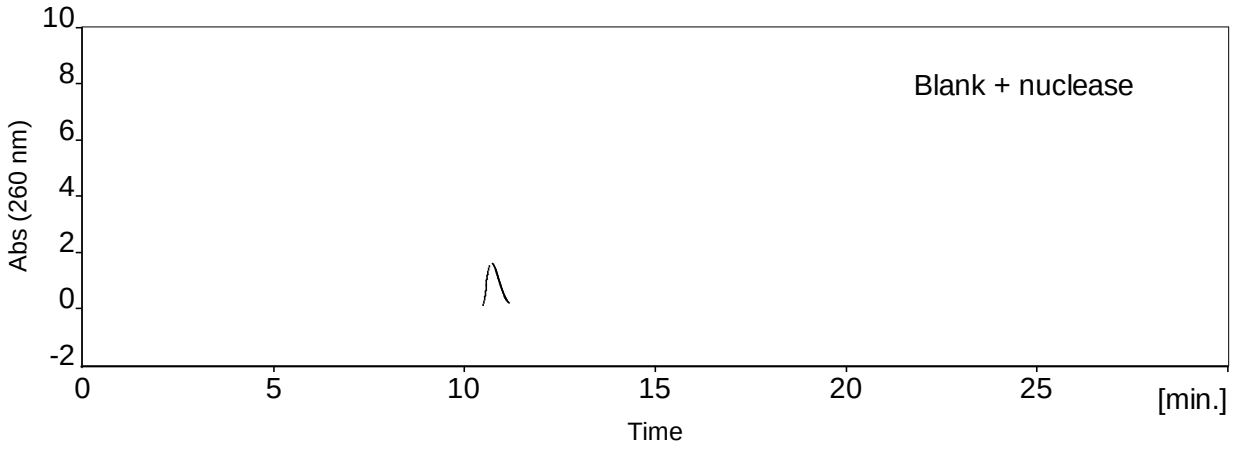
RP HPLC profile of ODN **25** that went through catching by polymerization



RP HPLC profile of nucleosides from ODN enzymatic digestion essay for ODNs that do not contain 8-oxo-dG—the blank control



RP HPLC profile of nucleosides from ODN enzymatic digestion essay for ODN that contain 8-oxo-dG—the blank control; the peak at 11 minutes is resulted from 2-mercaptoethanol added to prevent oxidation of 8-oxo-dG



Nucleoside peak areas in HPLC profiles of SVP and BAP digested ODNs

ODN	**20**				Authentic **20**				**24**				
Base	dC	dG	dT	dA	dC	dG	dT	dA	dC	dG	dT	dA	8-Oxo-dG
Number of base	6	4	7	3	6	4	7	3	6	3	7	3	1
Calcd relative area*	6.0	6.4	8.5	6.3	6.0	6.4	8.5	6.3	6.0	4.8	8.5	6.3	-
Meaured relative area	6.0	7.1	7.8	5.7	6.0	7.0	7.5	5.6	6.0	5.3	7.6	6.3	1.1

* Calculated based on the relative nucleoside extinction coefficients of dC/dG/dT/dA 1.0:1.6:1.2:2.1 from Current Protocols in Nucleic Acid Chemistry, Unit 10.6, DOI: 10.1002/0471142700.nc1006s01.

Recipes for the preparation of polymerization solutions

Cross-linking ratio	*N*,*N*′-Methylenebis(acrylamide)		*N*,*N*-Dimethylacrylamide		Water (mL)
	Mass (mmol)	Weight (mg)	Mass (mmol)	Volume (μL)	
1:50	0.169	26.05	8.44	870	5
1:25	0.337	52.1	8.44	870	5
1:15	0.563	86.8	8.44	870	5
1:7	1.207	186.1	8.44	870	5
1:2	4.225	651.3	8.44	870	14*

* More water had to be added to dissolve the materials.

ODN synthetic cycles using polymerizable phosphoramidite as capping agent

Cycle 1 — 0.2 µmol synthesis, the general ODN synthesis procedure

ODN synthetic cycle using polymerizable phosphoramidite as the capping agent
Synthesizer: standard ABI 394 solid phase synthesizer; 4-column 8-base instrument
Polymerizable capping agent: 0.15 M solution of capping phosphoramidite 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 steps
Synthesis scale: 0.2 µmol
Column used: column 2; functions for other columns are not shown

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.	111	Block vent	2.0
9.	58	Tet to waste	1.7
10.	33	B+Tet to column	2.0
11.	34	Tet to column	1.0
12.	33	B+Tet to column	1.5
13.	43	Push to column	
14.	143	Column 2 off	
15.	103	wait	25.0
16.	64	18 To waste	3.0
17.	42	18 To column	10.0
18.	2	Reverse flush	8.0
19.	1	Block flush	4.0
20.	101	Phos Prep	3.0
21.	142	Column 2 on	
22.	111	Block vent	2.0
23.	58	Tet to waste	1.7
24.	35	5+Tet to column	2.0
25.	34	Tet to column	1.0

26.	103	Wait	90.0
27.	35	5+Tet to column	1.5
28.	103	Wait	90.0
29.	35	5+Tet to column	1.5
30.	103	Wait	90.0
31.	35	5+Tet to column	1.5
32.	103	Wait	90.0
33.	43	Push to column	
34.	103	Wait	30.0
35.	64	18 To waste	4.0
36.	42	18 To column	8.0
37.	4	Flush to waste	4.0
38.	42	18 To column	8.0
39.	2	Reverse flush	5.0
40.	42	18 To column	8.0
41.	2	Reverse flush	5.0
42.	1	Block flush	3.0
43.	41	15 To column	8.0
44.	64	18 To waste	4.0
45.	1	Block flush	3.0
46.	103	Wait	10.0
47.	41	15 To column	8.0
48.	64	18 To waste	4.0
49.	1	Block flush	3.0
50.	103	Wait	10.0
51.	42	18 To column	15.0
52.	4	Flush to waste	4.0
53.	42	18 To column	10.0
54.	2	Reverse flush	5.0
55.	42	18 To column	10.0
56.	2	Reverse flush	5.0
57.	1	Block flush	3.0
58.	105	Start detrityl	
59.	64	18 To waste	4.0
60.	42	18 To column	10.0

61.	2	Reverse flush	5.0
62.	1	Block flush	3.0
63.	167	If monitoring	
64.	44	19 To column	25.0
65.	40	14 To column	3.0
66.	135	Monitor triyls	
67.	40	14 To column	25.0
68.	136	Monitor noise	
69.	40	14 To column	10.0
70.	137	Stop monitor	
71.	42	18 To column	10.0
72.	2	Reverse flush	8.0
73.	168	If not monitoring	
74.	40	14 To column	6.0
75.	3	Trityl flush	5.0
76.	40	14 To column	6.0
77.	103	Wait	5.0
78.	3	Trityl flush	5.0
79.	40	14 To column	6.0
80.	103	Wait	5.0
81.	3	Trityl flush	5.0
82.	40	14 To column	6.0
83.	103	Wait	5.0
84.	3	Trityl flush	5.0
85.	42	18 To column	10.0
86.	3	Trityl flush	8.0
87.	169	End monitoring	
88.	42	18 To column	8.0
89.	2	Reverse flush	5.0
90.	1	Block flush	4.0
91.	107	End	

Note: Bottle 5—polymerizable capping phosphoramidite; bottle 14—detritylation solution; bottle 15—oxidation solution; bottle 18—acetonitrile; bottle 19—dichloromethane; B—base or phosphoramidite; Tet—tetrazole or activator.

Cycle 2 — 0.2 µmol synthesis, the general ODN synthesis procedure with shortened capping time

ODN synthetic cycle using polymerizable phosphoramidite as the capping agent
Synthesizer: standard ABI 394 solid phase synthesizer; 4-column 8-base instrument
Polymerizable capping agent: 0.15 or 0.1 M solution of polymerizable phosphoramidite 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; functions for other columns are not shown

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.	111	Block vent	2.0
9.	58	Tet to waste	1.7
10.	33	B+Tet to column	2.0
11.	34	Tet to column	1.0
12.	33	B+Tet to column	1.5
13.	43	Push to column	
14.	143	Column 2 off	
15.	103	wait	25.0
16.	64	18 To waste	3.0
17.	42	18 To column	10.0
18.	2	Reverse flush	8.0
19.	1	Block flush	4.0
20.	101	Phos Prep	3.0
21.	142	Column 2 on	
22.	111	Block vent	2.0
23.	58	Tet to waste	1.7
24.	35	5+Tet to column	2.0
25.	34	Tet to column	1.0

26.	103	Wait	30.0
27.	35	5+Tet to column	1.5
28.	103	Wait	30.0
29.	35	5+Tet to column	1.5
30.	103	Wait	30.0
31.	43	Push to column	
32.	103	Wait	30.0
33.	64	18 To waste	4.0
34.	42	18 To column	8.0
35.	4	Flush to waste	4.0
36.	42	18 To column	8.0
37.	2	Reverse flush	5.0
38.	42	18 To column	8.0
39.	2	Reverse flush	5.0
40.	1	Block flush	3.0
41.	41	15 To column	8.0
42.	64	18 To waste	4.0
43.	1	Block flush	3.0
44.	103	Wait	10.0
45.	41	15 To column	8.0
46.	64	18 To waste	4.0
47.	1	Block flush	3.0
48.	103	Wait	10.0
49.	42	18 To column	15.0
50.	4	Flush to waste	4.0
51.	42	18 To column	10.0
52.	2	Reverse flush	5.0
53.	42	18 To column	10.0
54.	2	Reverse flush	5.0
55.	1	Block flush	3.0
56.	105	Start detrityl	
57.	64	18 To waste	4.0
58.	42	18 To column	10.0
59.	2	Reverse flush	5.0
60.	1	Block flush	3.0

61.	167	If monitoring	
62.	44	19 To column	25.0
63.	40	14 To column	3.0
64.	135	Monitor triyls	
65.	40	14 To column	25.0
66.	136	Monitor noise	
67.	40	14 To column	10.0
68.	137	Stop monitor	
69.	42	18 To column	10.0
70.	2	Reverse flush	8.0
71.	168	If not monitoring	
72.	40	14 To column	6.0
73.	3	Trityl flush	5.0
74.	40	14 To column	6.0
75.	103	Wait	5.0
76.	3	Trityl flush	5.0
77.	40	14 To column	6.0
78.	103	Wait	5.0
79.	3	Trityl flush	5.0
80.	40	14 To column	6.0
81.	103	Wait	5.0
82.	3	Trityl flush	5.0
83.	42	18 To column	10.0
84.	3	Trityl flush	8.0
85.	169	End monitoring	
86.	42	18 To column	8.0
87.	2	Reverse flush	5.0
88.	1	Block flush	4.0
89.	107	End	

Note: Bottle 5—polymerizable capping phosphoramidite; bottle 14—detritylation solution; bottle 15—oxidation solution; bottle 18—acetonitrile; bottle 19—dichloromethane; B—base or phosphoramidite; Tet—tetrazole or activator.

Cycle 3 — 1.0 µmol synthesis

ODN synthetic cycle using polymerizable phosphoramidite as the capping agent
Synthesizer: standard ABI 394 solid phase synthesizer; 4-column 8-base instrument
Polymerizable capping agent: 0.15 M solution of polymerizable phosphoramidite in acetonitrile unless otherwise noted in the article, 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: 1.0 µmol
Column used: column 2; functions for other columns are not shown

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	10.0
5.	1	Block flush	4.0
6.	101	Phos Prep	3.0
7.	142	Column 2 on	
8.	111	Block vent	2.0
9.	58	Tet to waste	1.7
10.	33	B+Tet to column	2.5
11.	34	Tet to column	1.0
12.	33	B+Tet to column	2.5
13.	43	Push to column	
14.	143	Column 2 off	
15.	103	wait	50.0
16.	64	18 To waste	3.0
17.	42	18 To column	10.0
18.	4	Flush to waste	5.0
19.	42	18 To column	10.0
20.	2	Reverse flush	10.0
21.	1	Block flush	4.0
22.	101	Phos Prep	3.0
23.	142	Column 2 on	
24.	111	Block vent	2.0
25.	58	Tet to waste	1.7
26.	35	5+Tet to column	2.5

27.	34	Tet to column	1.0
28.	103	Wait	30.0
29.	35	5+Tet to column	2.5
30.	103	Wait	30.0
31.	35	5+Tet to column	2.5
32.	103	Wait	30.0
33.	43	Push to column	
34.	103	Wait	30.0
35.	64	18 To waste	4.0
36.	42	18 To column	10.0
37.	4	Flush to waste	6.0
38.	42	18 To column	10.0
39.	4	Flush to waste	6.0
40.	42	18 To column	10.0
41.	2	Reverse flush	7.0
42.	1	Block flush	3.0
43.	41	15 To column	8.0
44.	64	18 To waste	4.0
45.	1	Block flush	3.0
46.	103	Wait	15.0
47.	41	15 To column	8.0
48.	64	18 To waste	4.0
49.	1	Block flush	3.0
50.	103	Wait	15.0
51.	42	18 To column	15.0
52.	4	Flush to waste	6.0
53.	42	18 To column	10.0
54.	4	Flush to waste	6.0
55.	42	18 To column	10.0
56.	2	Reverse flush	7.0
57.	1	Block flush	3.0
58.	105	Start detrityl	
59.	64	18 To waste	4.0
60.	42	18 To column	10.0
61.	2	Reverse flush	5.0

62.	1	Block flush	3.0
63.	167	If monitoring	
64.	44	19 To column	25.0
65.	40	14 To column	3.0
66.	135	Monitor triyls	
67.	40	14 To column	25.0
68.	136	Monitor noise	
69.	40	14 To column	10.0
70.	137	Stop monitor	
71.	42	18 To column	10.0
72.	2	Reverse flush	8.0
73.	168	If not monitoring	
74.	40	14 To column	6.0
75.	3	Trityl flush	5.0
76.	40	14 To column	6.0
77.	103	Wait	5.0
78.	3	Trityl flush	5.0
79.	40	14 To column	6.0
80.	103	Wait	5.0
81.	3	Trityl flush	5.0
82.	40	14 To column	6.0
83.	103	Wait	5.0
84.	3	Trityl flush	5.0
85.	42	18 To column	10.0
86.	3	Trityl flush	8.0
87.	169	End monitoring	
88.	42	18 To column	8.0
89.	2	Reverse flush	5.0
90.	1	Block flush	4.0
91.	107	End	

Note: Bottle 5—polymerizable capping phosphoramidite; bottle 14—detritylation solution; bottle 15—oxidation solution; bottle 18—acetonitrile; bottle 19—dichloromethane; B—base or phosphoramidite; Tet—tetrazole or other activator.