Electronic Supporting Information (ESI) for *New Journal of Chemistry*

Oligonucleotide synthesis under mild deprotection conditions

Komal Chillar[1,2,+], Adikari M. D. N. Eriyagama[1,2,+], Yipeng Yin[1,2,+], Shahien Shahsavari[1,2,+], Bhaskar Halami[1,1], Alexander Apostle[1,2], Shiyue Fang[1,2,*]

[1] Department of Chemistry, Michigan Technological University, 1400 Townsend Drive, Houghton, MI, 49931, USA
[2] Health Research Institute, Michigan Technological University, 1400 Townsend Drive, Houghton, MI, 49931, USA

[+] Equal contributors

* To whom correspondence should be addressed. Tel: 1-906-487-2023; Fax: 1-906-487-2061; Email: shifang@mtu.edu

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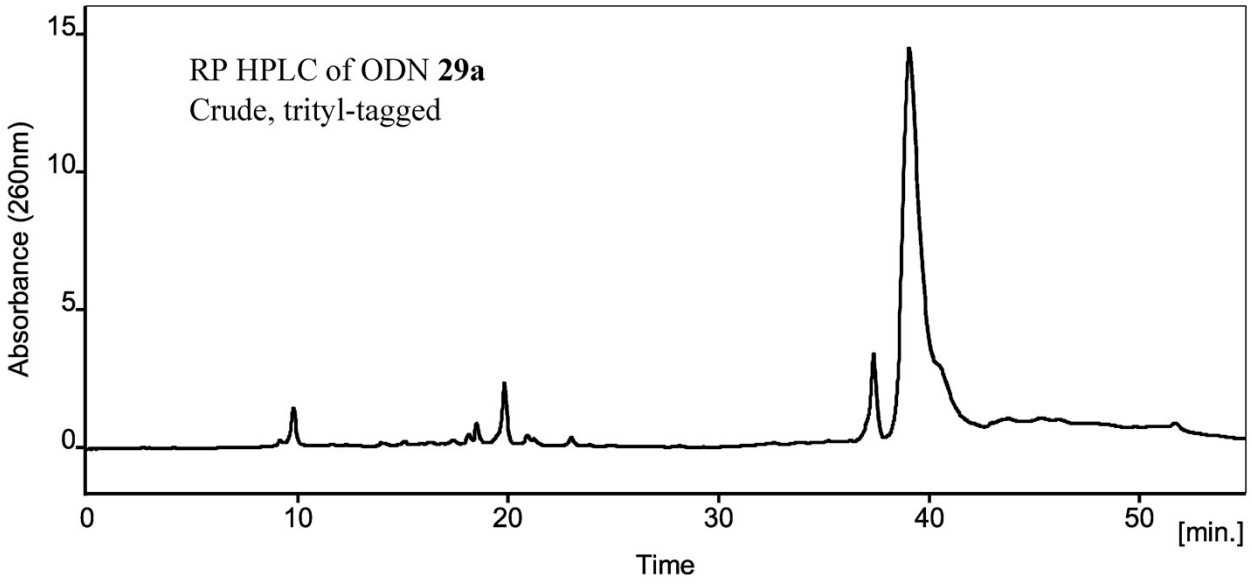
RP HPLC of ODN **29a**
Crude, trityl-tagged
Absorbance (260nm)
15
10
5
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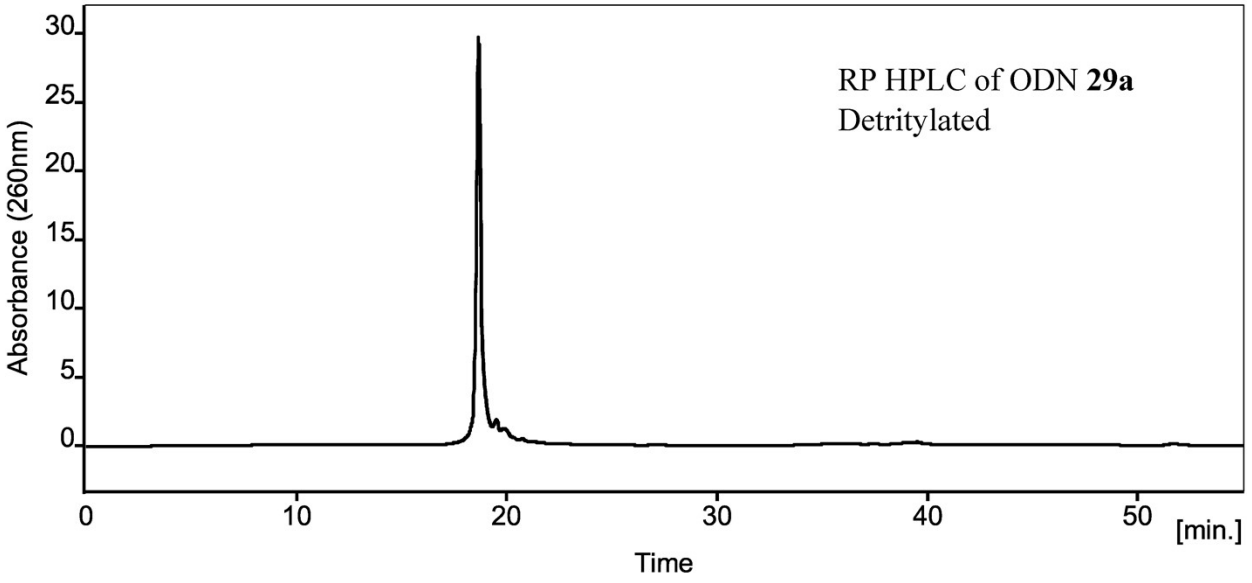
10
20
30
40
50
[min.]
Time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Detritylated
Absorbance (260nm)
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15
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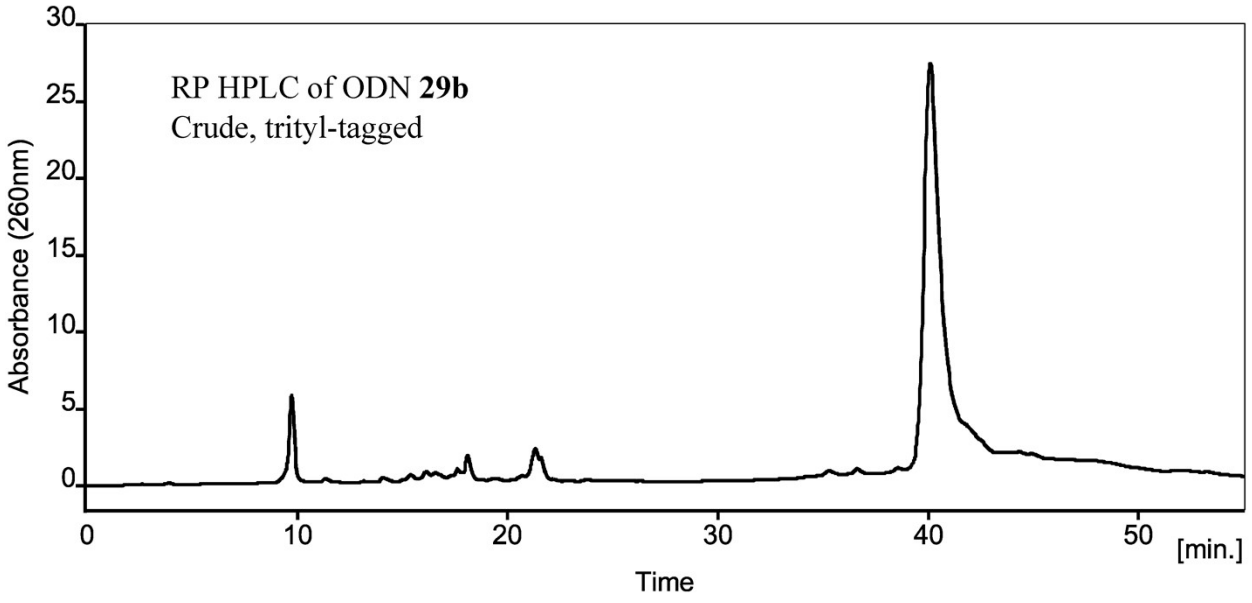
30
40
50
[min.]
Time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Crude, trityl-tagged
Absorbance (260nm)
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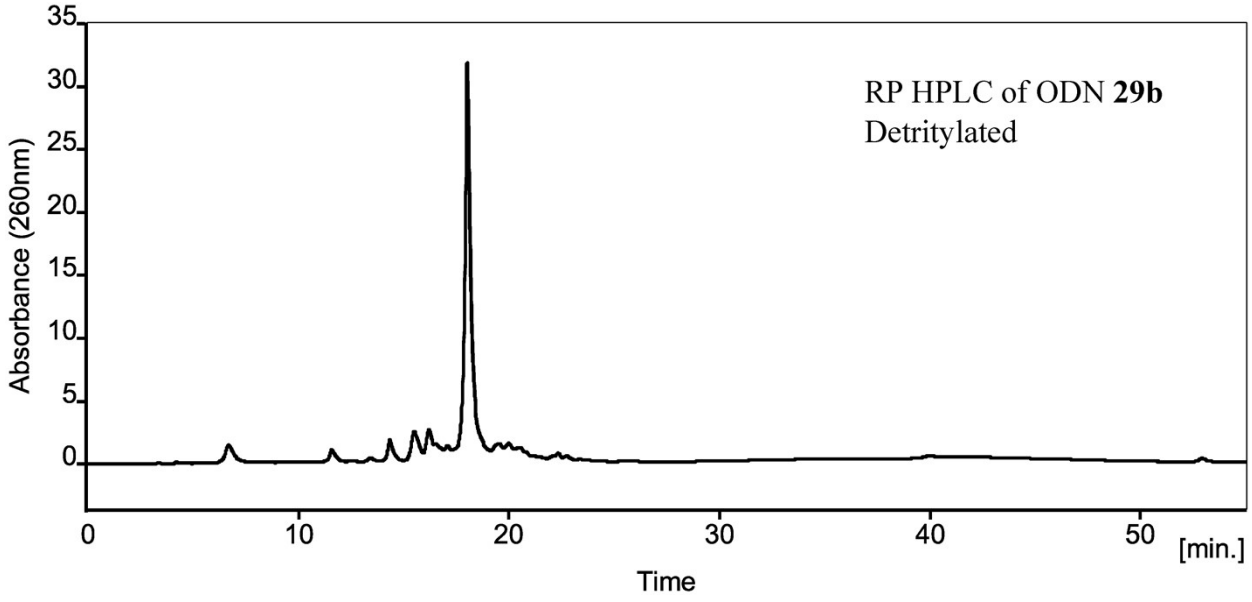
10
20
30
40
50
[min.]
Time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Detritylated
Absorbance (260nm)
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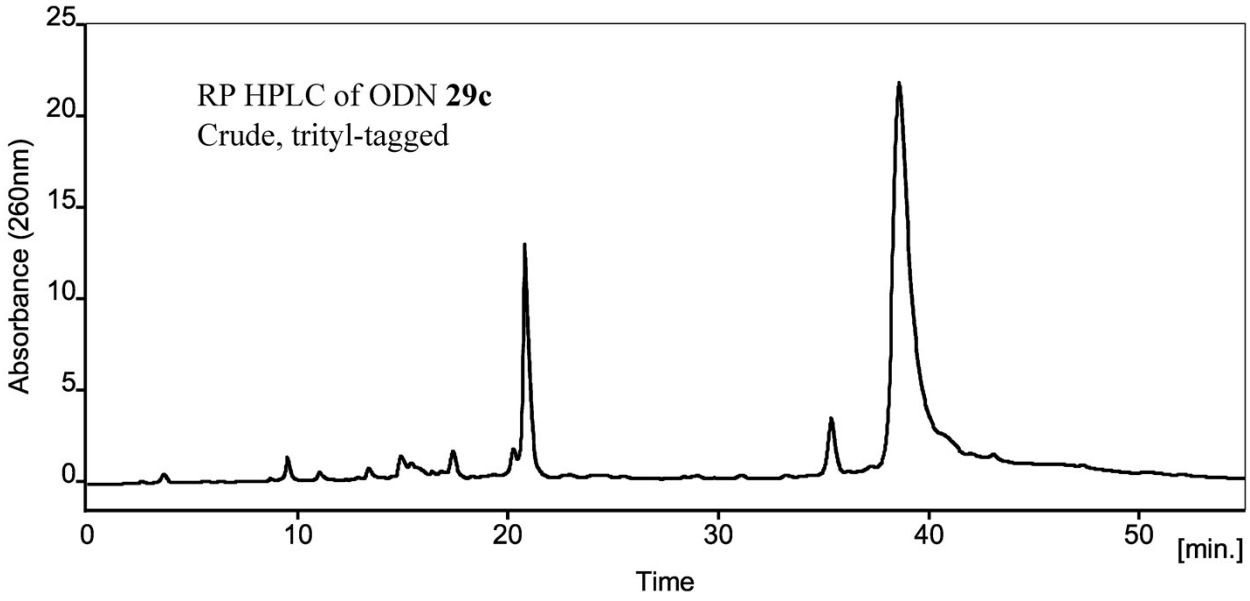
0
0
10
20
30
40
50
[min.]
Time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Crude, trityl-tagged
Absorbance (260nm)
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0
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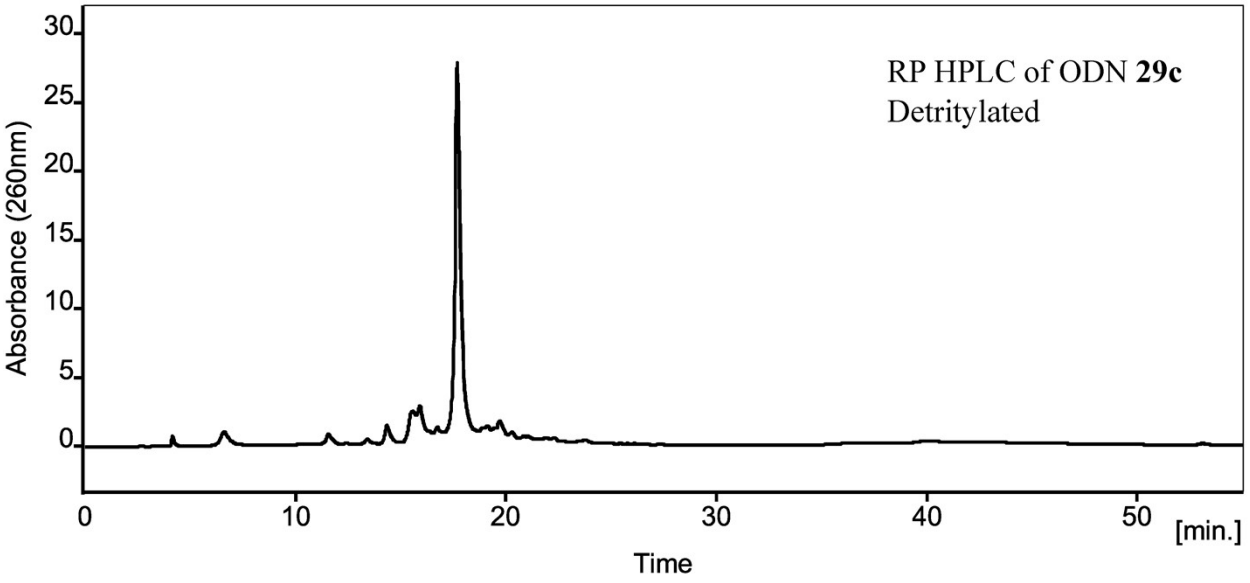
10
20
30
40
50
[min.]
Time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Detritylated
Absorbance (260nm)
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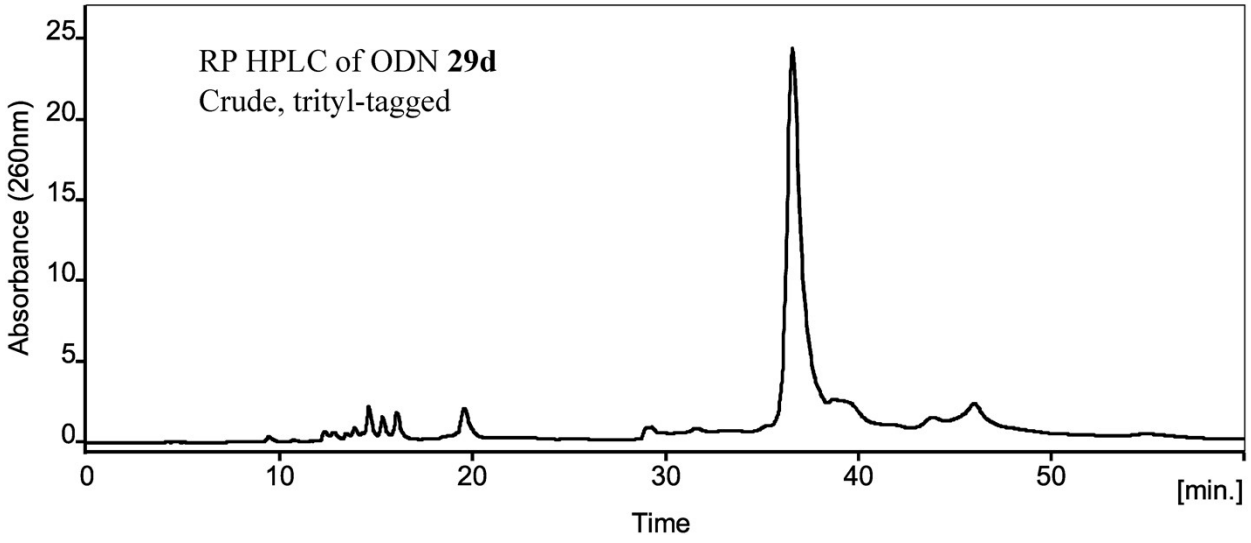
10
5
0
0
10
20
30
40
50
[min.]
Time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15
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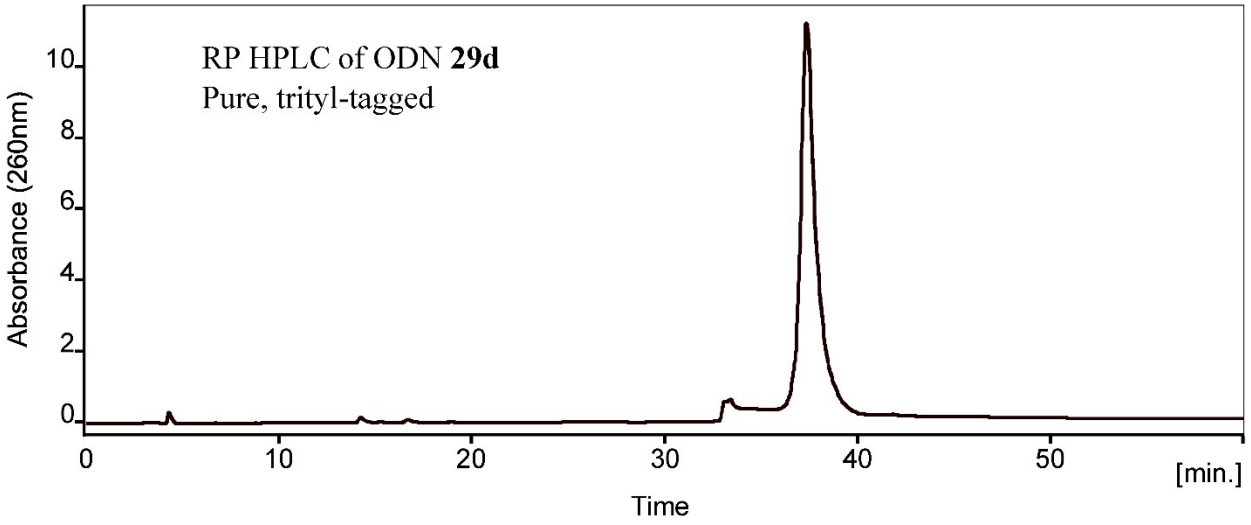
0
0
10
20
30
40
50
[min.]
Time


RP HPLC of ODN **29d**
Pure, trityl-tagged
Absorbance (260nm)
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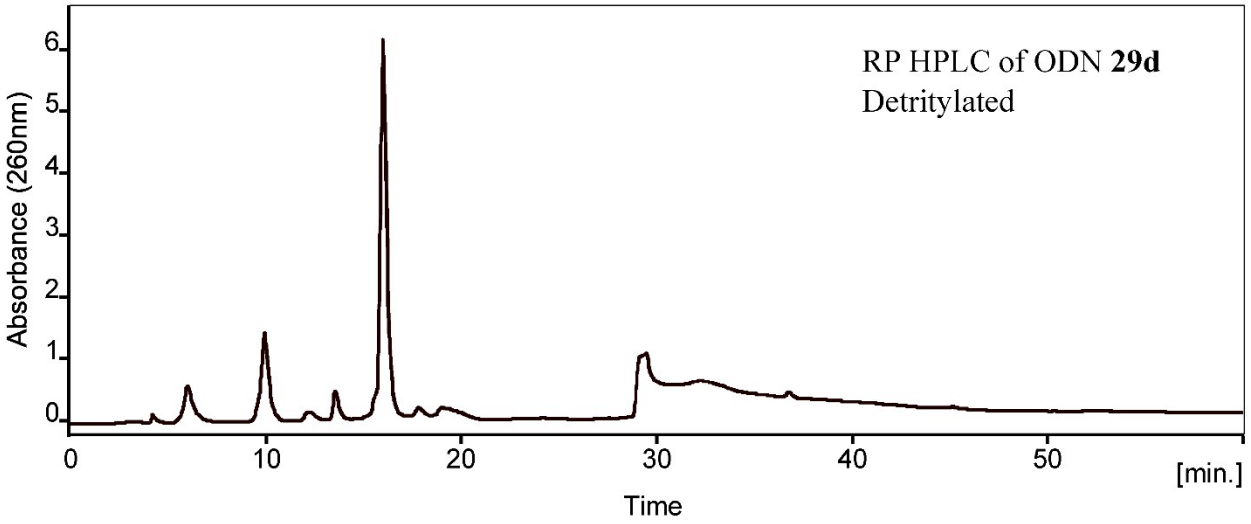
8
6
4
2
0
0
10
20
30
40
50
[min.]
Time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Detritylated
Absorbance (260nm)
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2
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0
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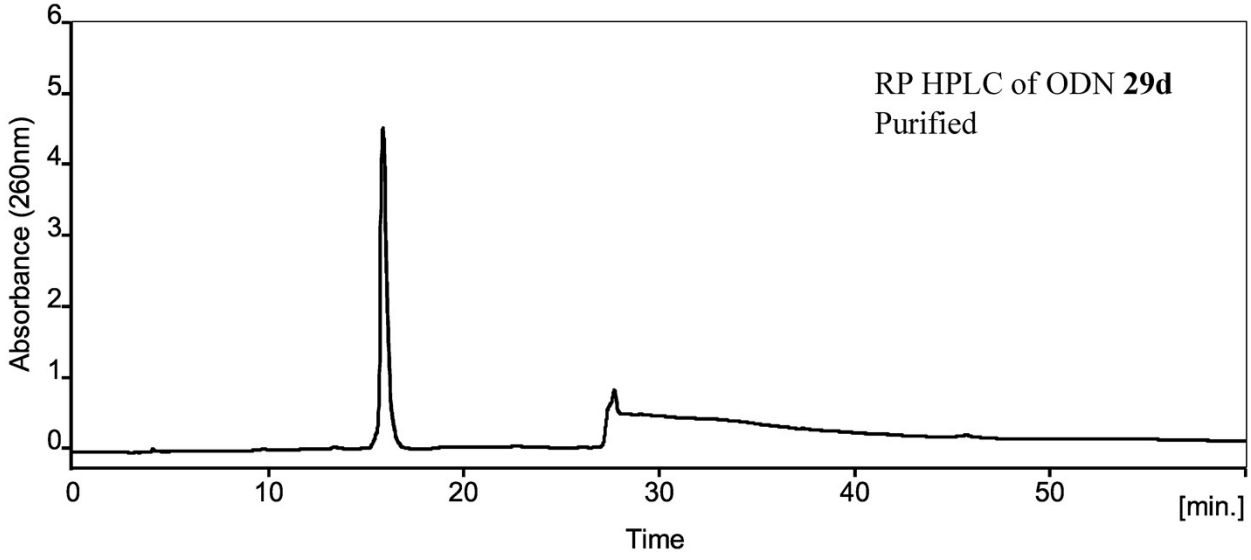
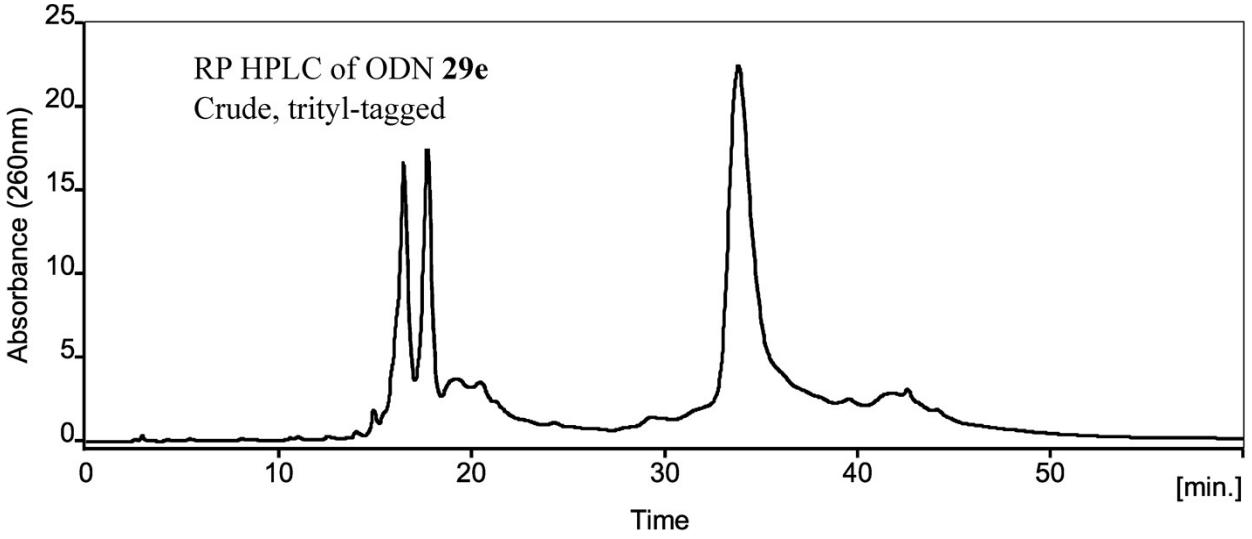
10
20
30
40
50
[min.]
Time


RP HPLC of ODN **29d**
Purified
Absorbance (260nm)
6
5
4
3
2
1
0
0
10
20
30
40
50
[min.]
Time


RP HPLC of ODN **29e**
Crude, trityl-tagged
Absorbance (260nm)
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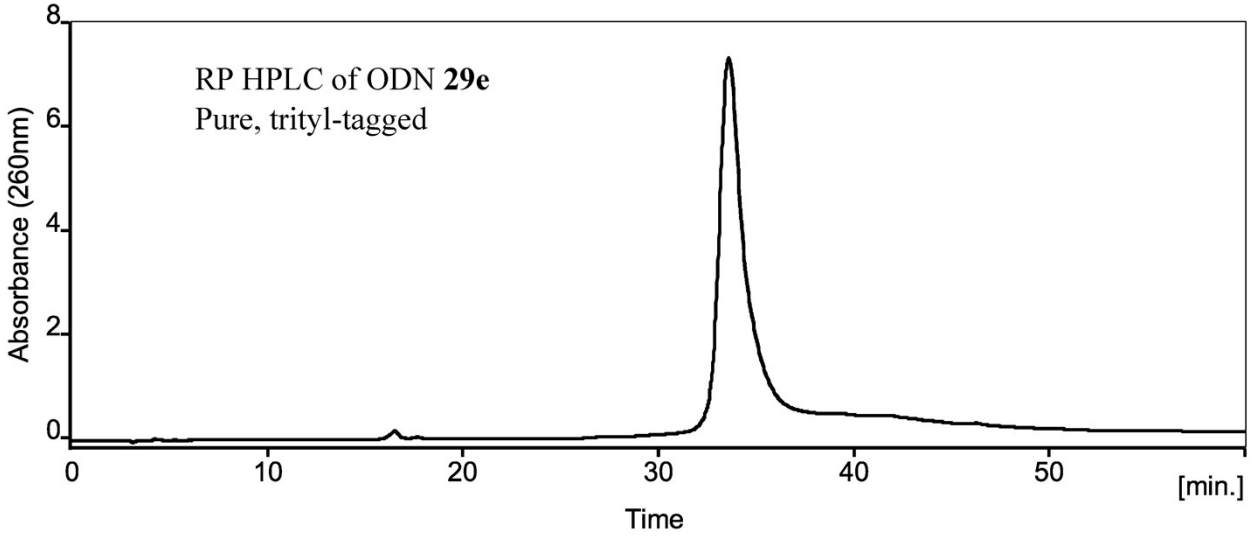
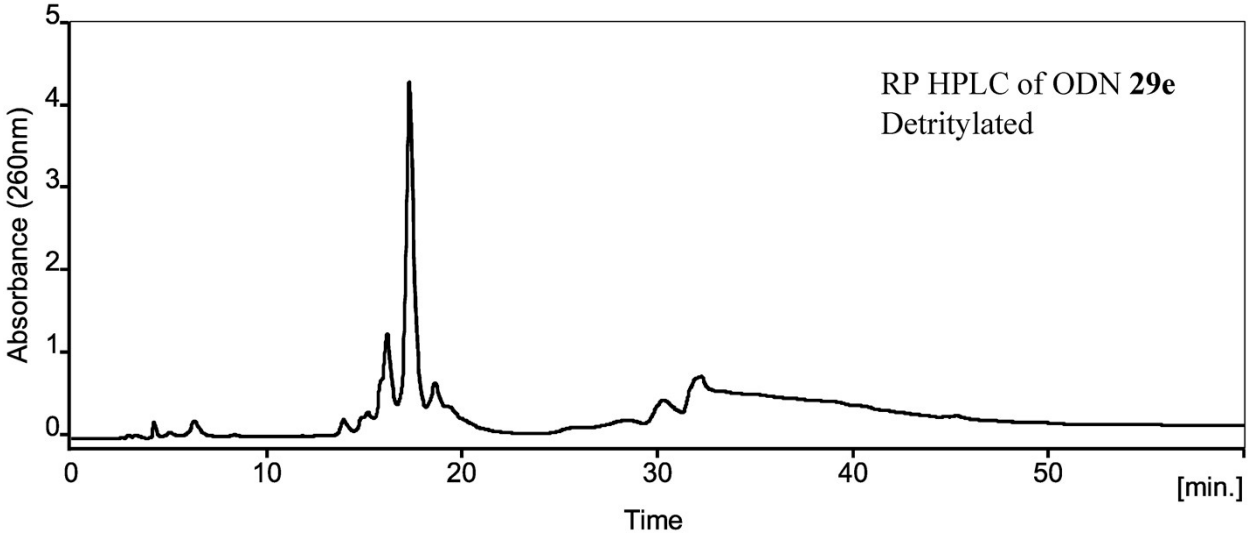
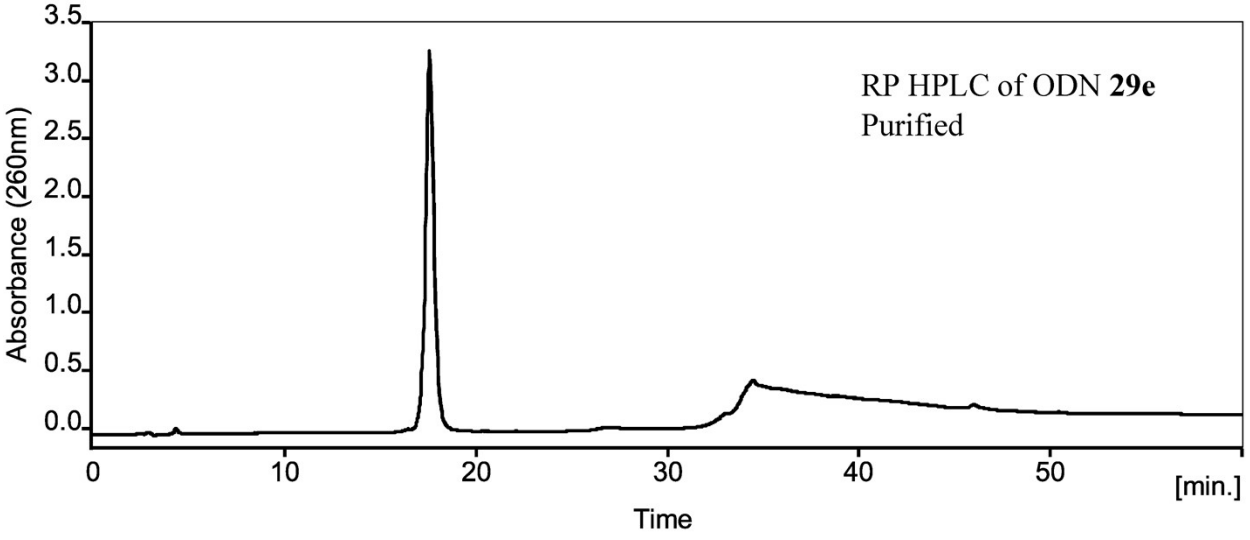
20
15
10
5
0
0
10
20
30
40
50
[min.]
Time


RP HPLC of ODN **29e**
Pure, trityl-tagged
Absorbance (260nm)
8
6
4
2
0
0
10
20
30
40
50
[min.]
Time


RP HPLC of ODN **29e**
Detritylated
Absorbance (260nm)
5
4
3
2
1
0
0
10
20
30
40
50
[min.]
Time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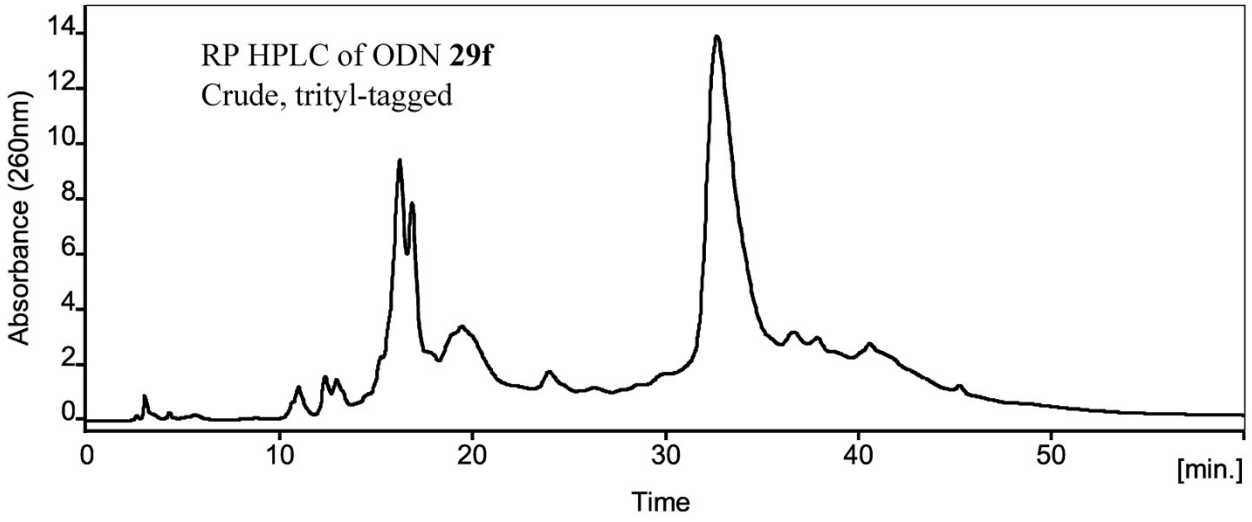
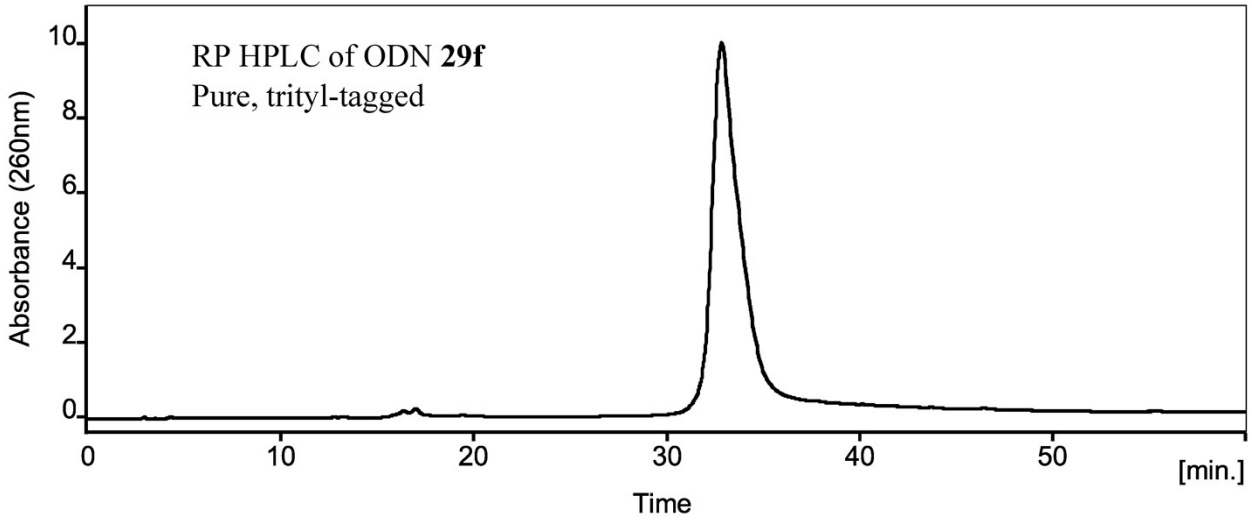
Purified
Absorbance (260nm)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
10
20
30
40
50
[min.]
Time


RP HPLC of ODN **29f**
Crude, trityl-tagged
Absorbance (260nm)
14
12
10
8
6
4
2
0
0
10
20
30
40
50
[min.]
Time


RP HPLC of ODN **29f**
Pure, trityl-tagged
Absorbance (260nm)
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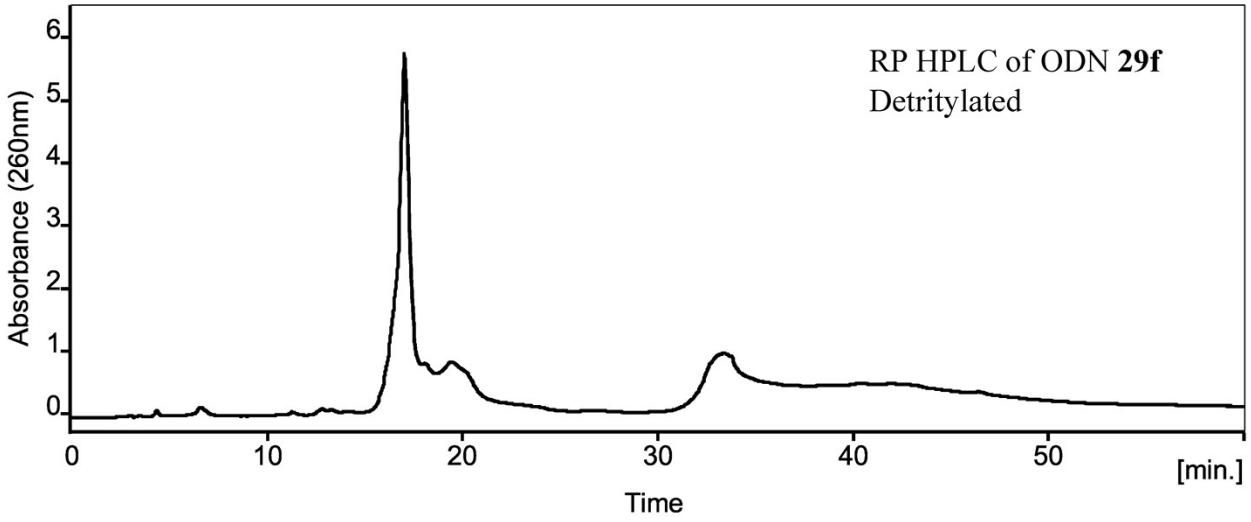
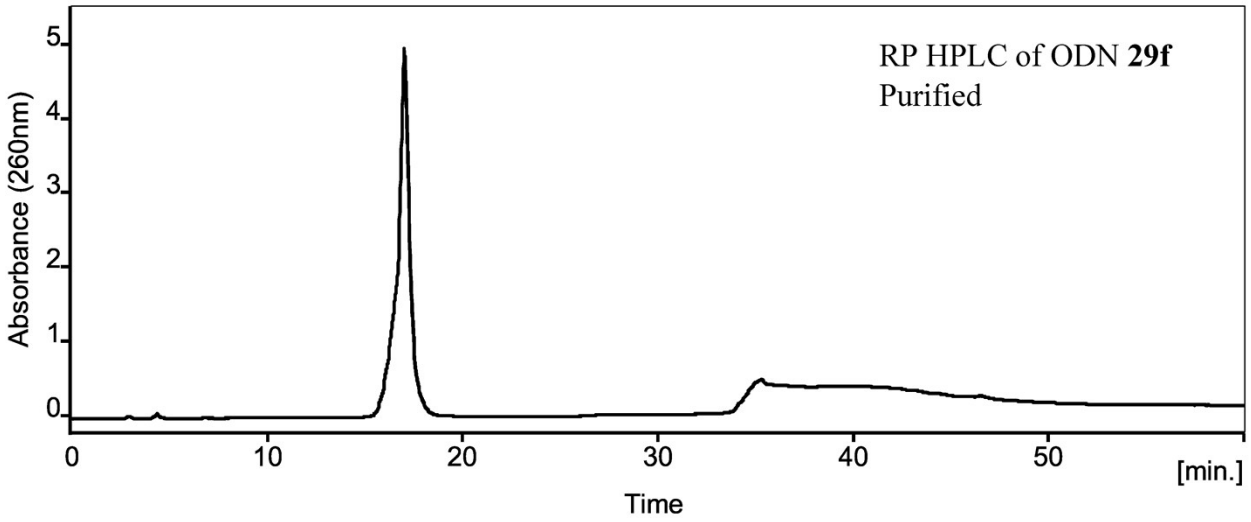
8
6
4
2
0
0
10
20
30
40
50
[min.]
Time


RP HPLC of ODN **29f**
Detritylated
Absorbance (260nm)
6
5
4
3
2
1
0
0
10
20
30
40
50
[min.]
Time


RP HPLC of ODN **29f**
Purified
Absorbance (260nm)
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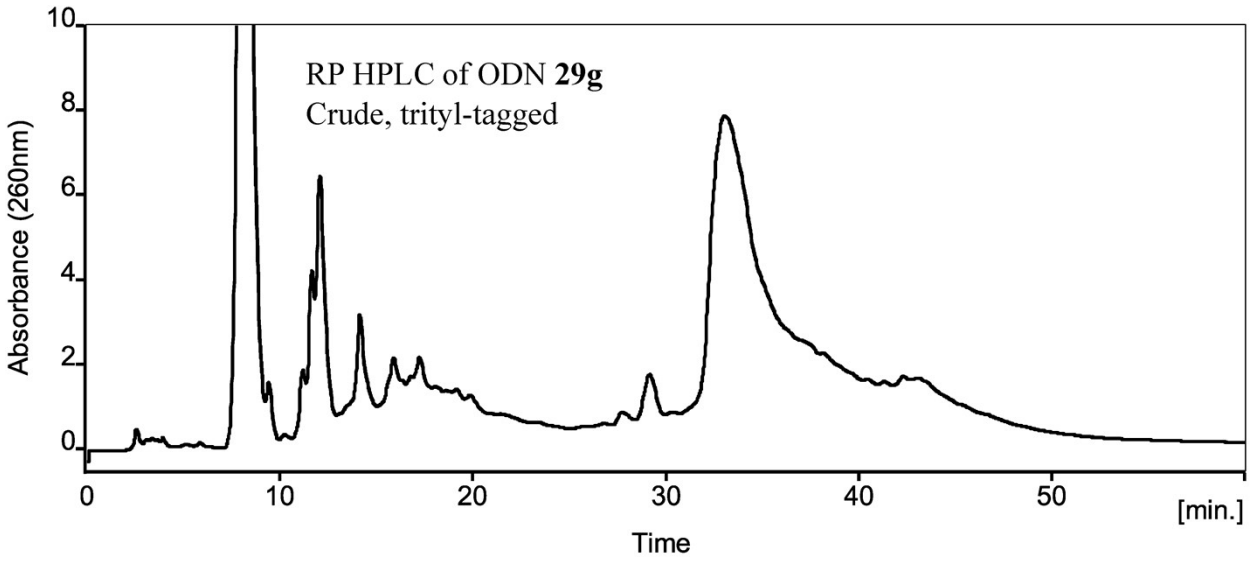
4
3
2
1
0
0
10
20
30
40
50
[min.]
Time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Crude, trityl-tagged
Absorbance (260nm)
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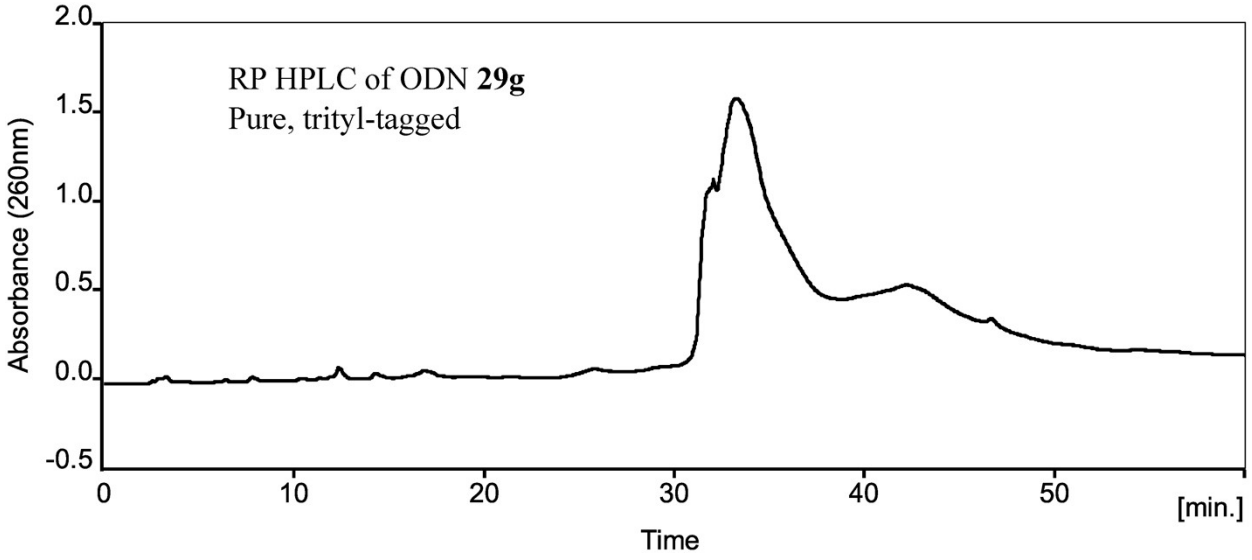
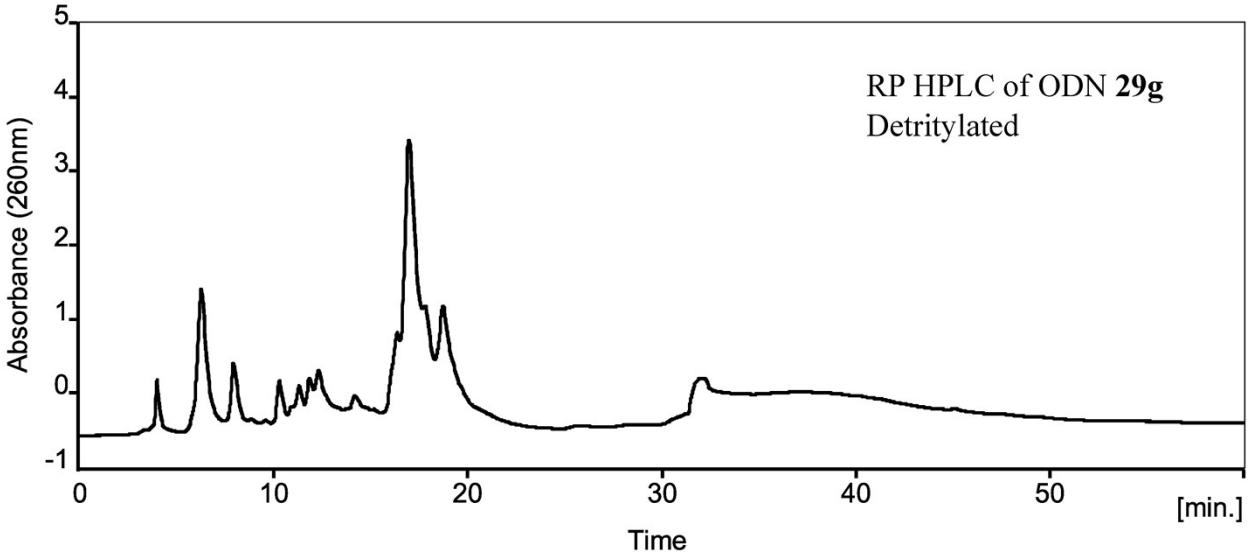
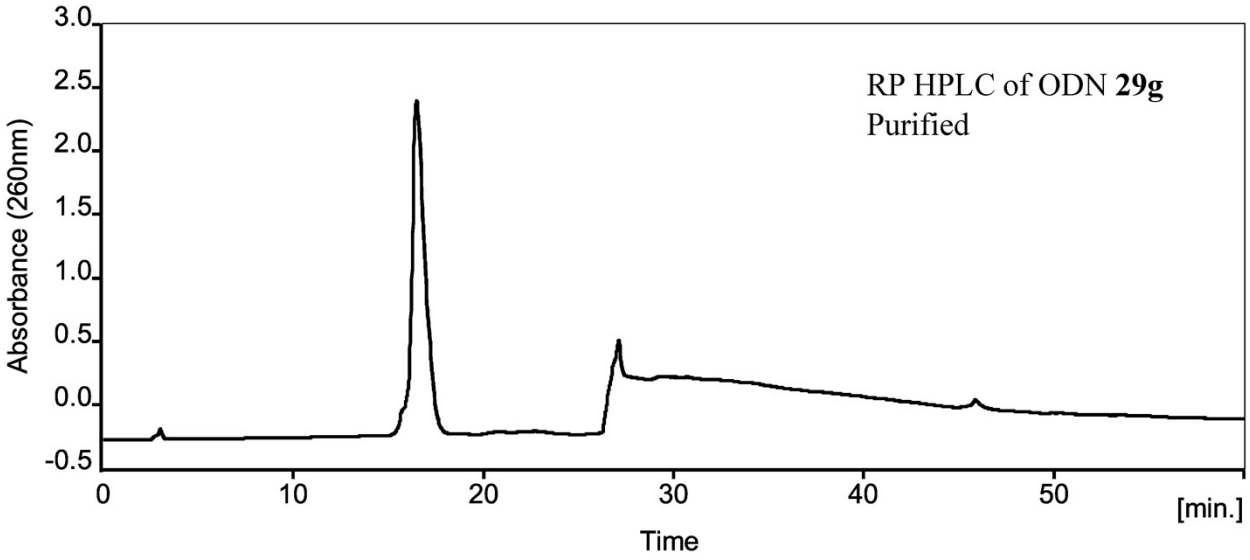
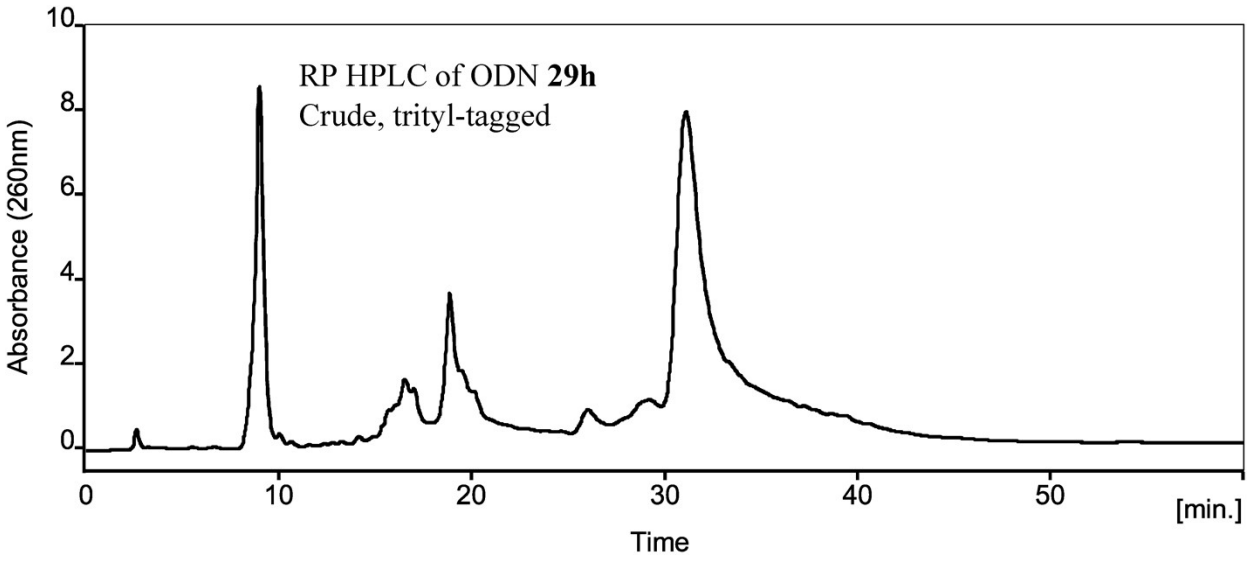
8
6
4
2
0
0
10
20
30
40
50
[min.]
Time


RP HPLC of ODN **29g**
Pure, trityl-tagged
Absorbance (260nm)
2.0
1.5
1.0
0.5
0.0
-0.5
0
10
20
30
40
50
[min.]
Time


RP HPLC of ODN **29g**
Detritylated
Absorbance (260nm)
5
4
3
2
1
0
-1
0
10
20
30
40
50
[min.]
Time


RP HPLC of ODN **29g**
Purified
Absorbance (260nm)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
0
10
20
30
40
50
[min.]
Time


RP HPLC of ODN **29h**
Crude, trityl-tagged
Absorbance (260nm)
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8
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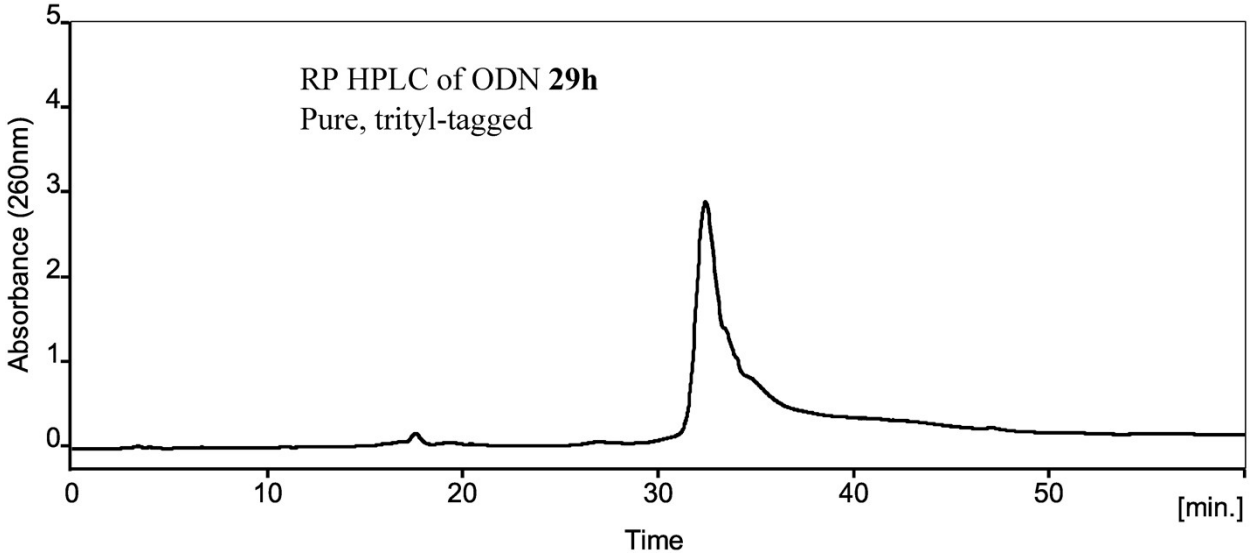
4
2
0
0
10
20
30
40
50
[min.]
Time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Absorbance (260nm)
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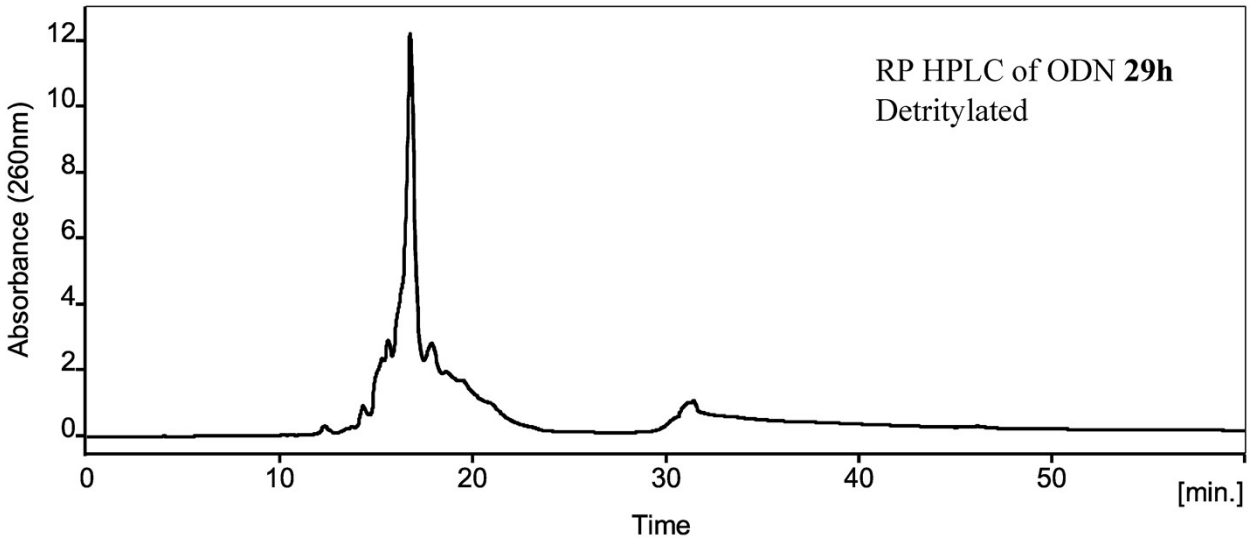
[min.]
Time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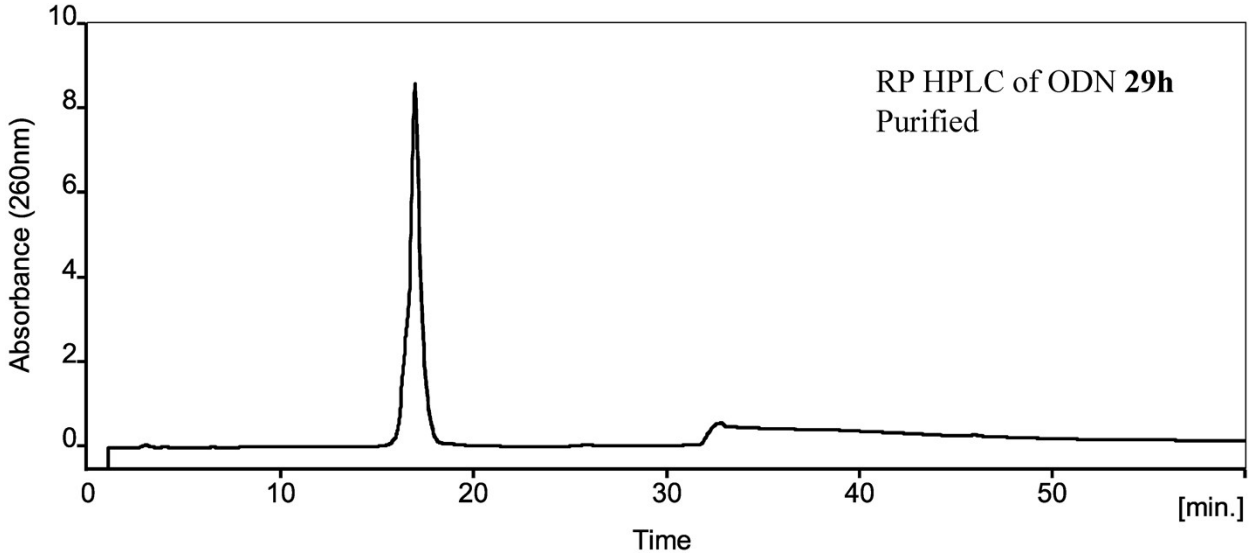
Detritylated
Absorbance (260nm)
12
10
8
6
4
2
0
0
10
20
30
40
50
[min.]
Time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Purified
Absorbance (260nm)
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8
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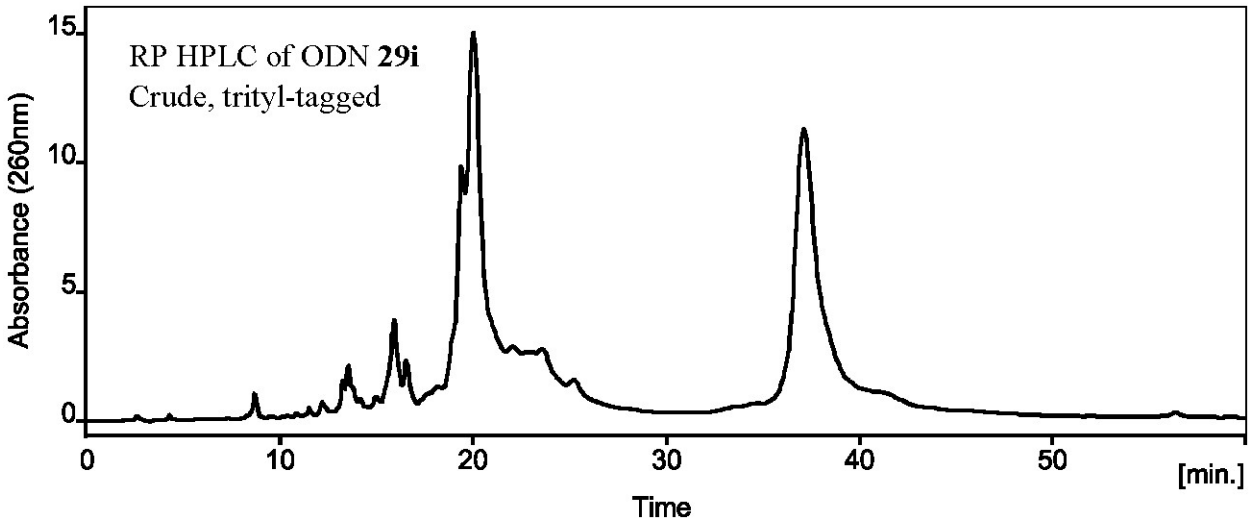
4
2
0
0
10
20
30
40
50
[min.]
Time


RP HPLC of ODN **29i**
Crude, trityl-tagged
Absorbance (260nm)
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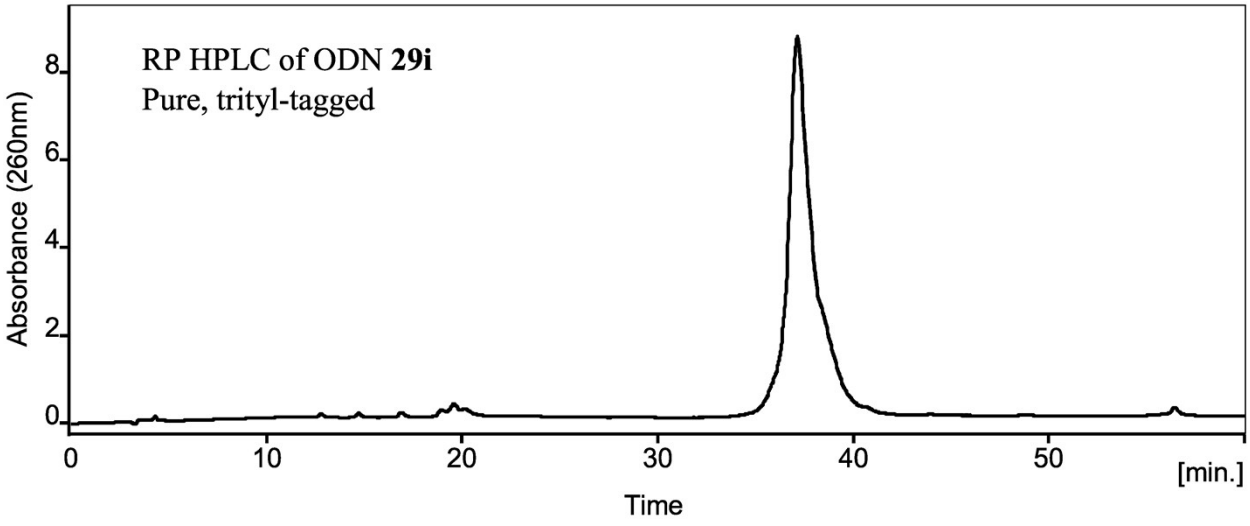
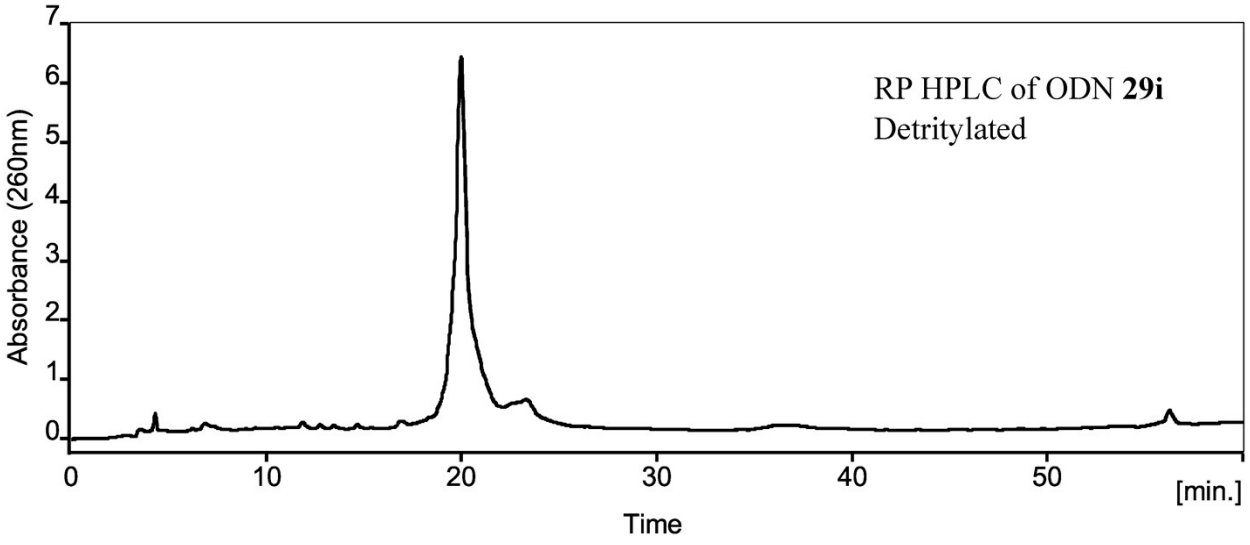
10
5
0
0
10
20
30
40
50
[min.]
Time


RP HPLC of ODN **29i**
Pure, trityl-tagged
Absorbance (260nm)
8
6
4
2
0
0
10
20
30
40
50
[min.]
Time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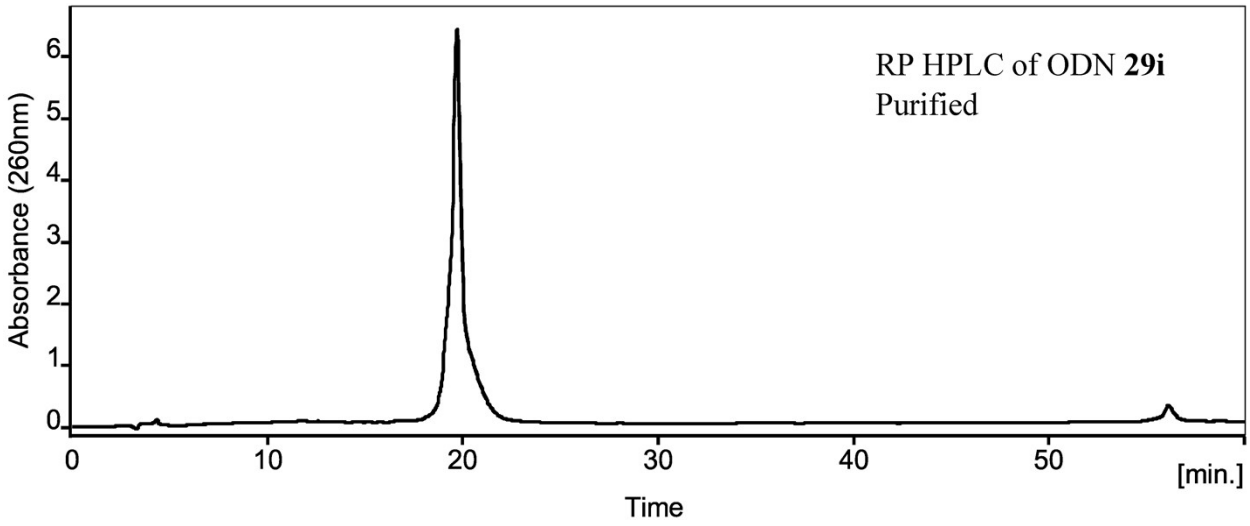
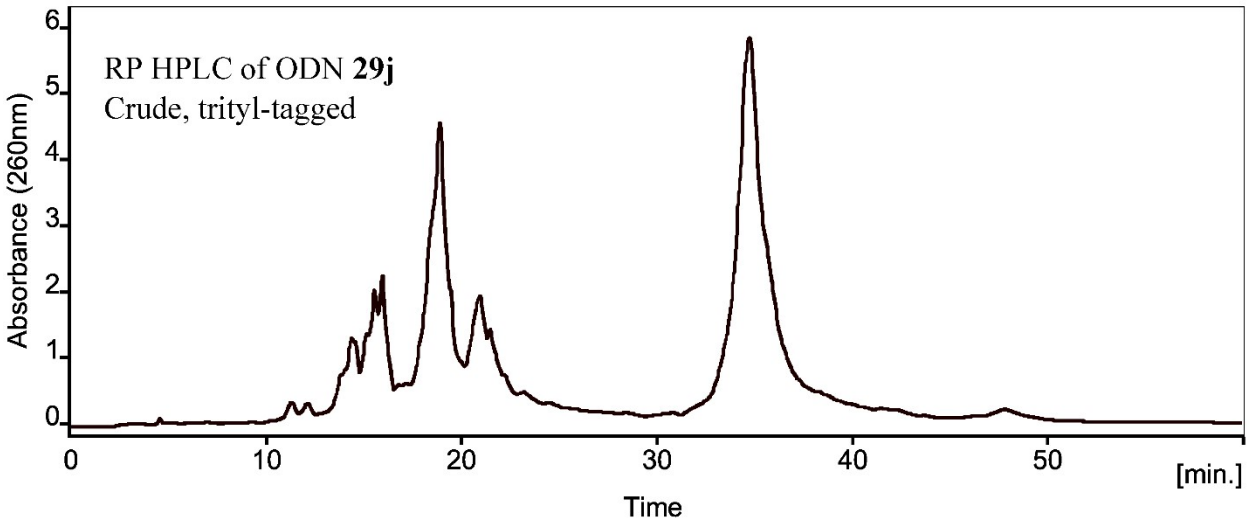
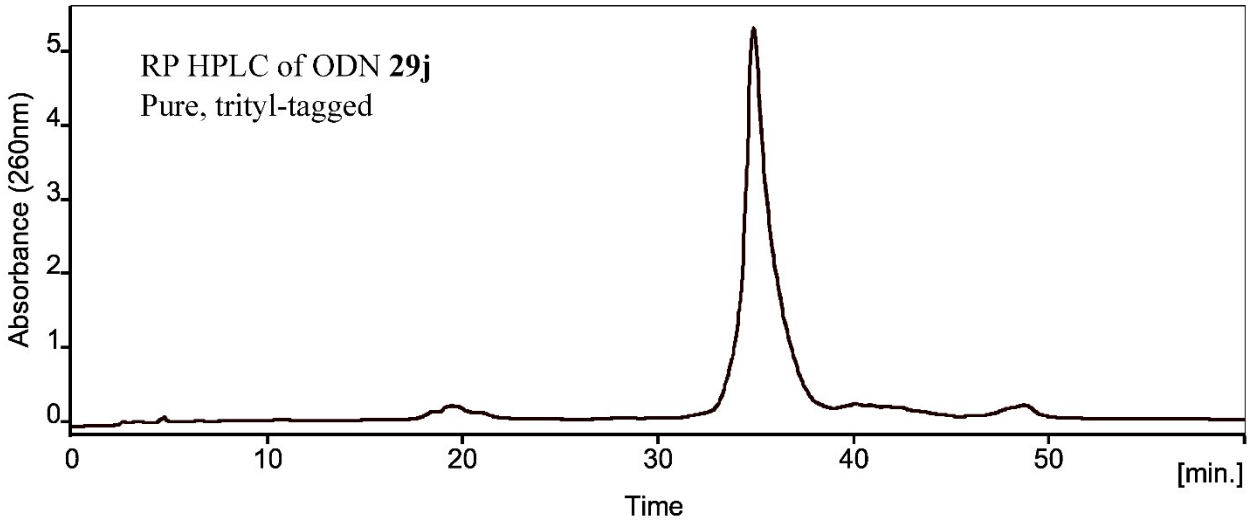
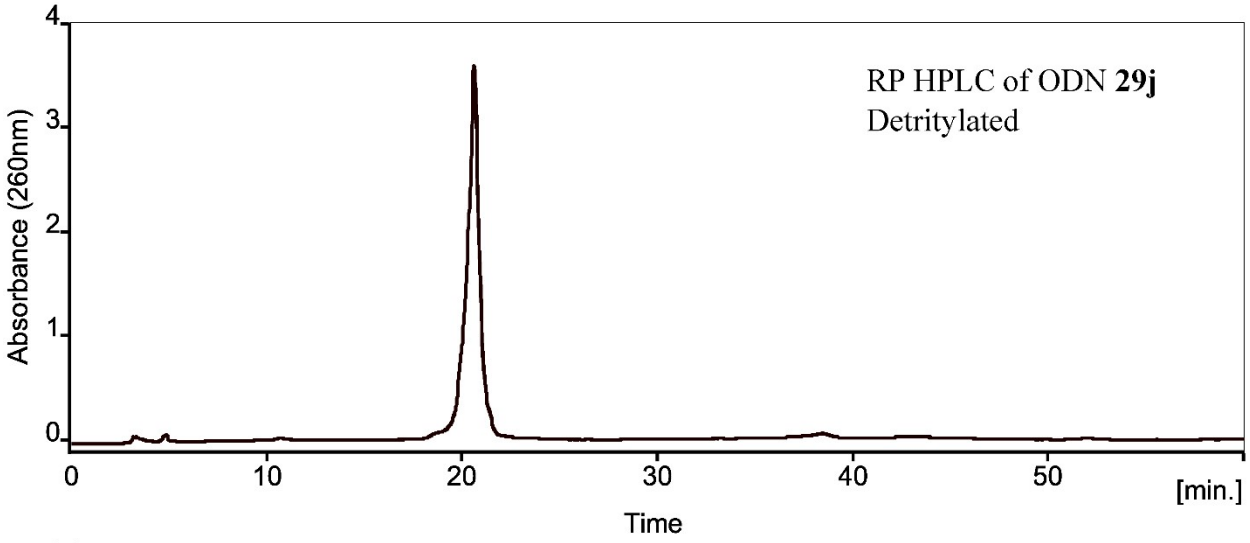
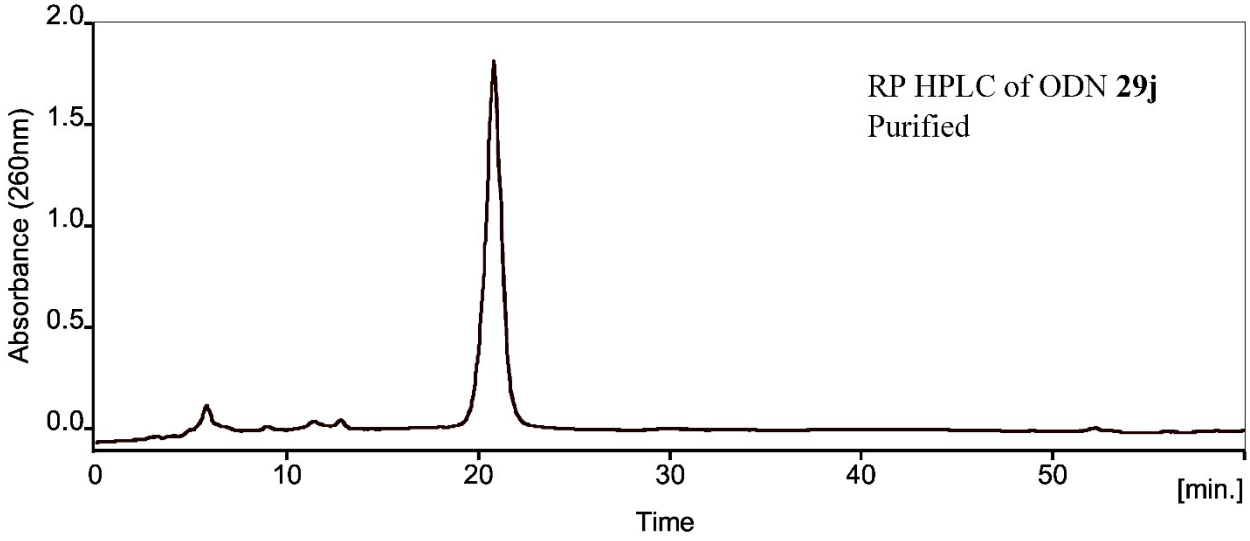
Detritylated
Absorbance (260nm)
Time
[min.]

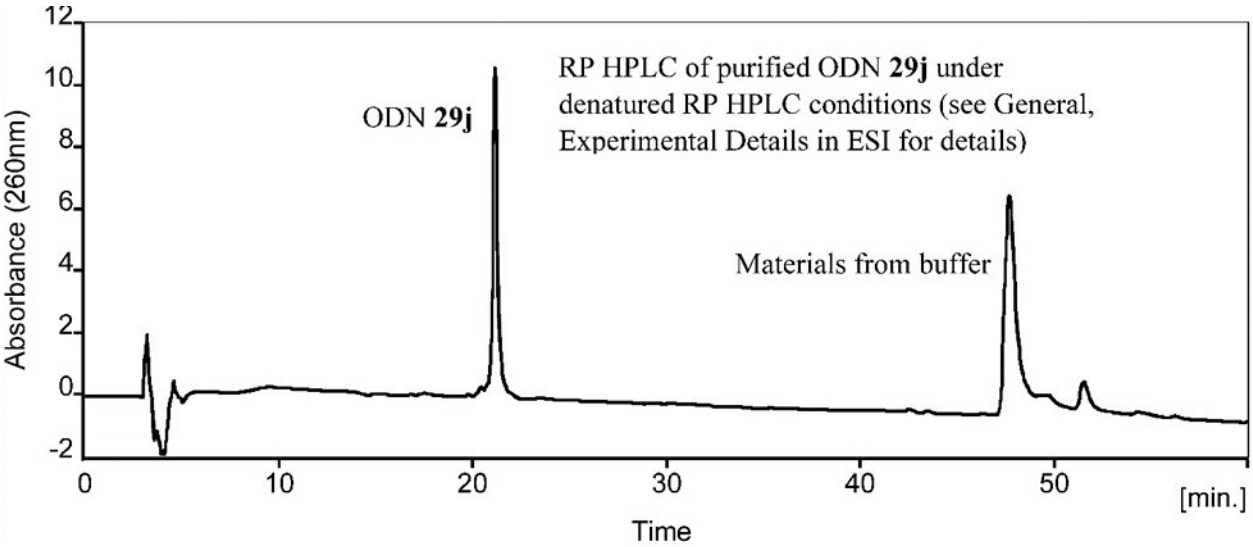

RP HPLC of ODN **29i**
Purified
Absorbance (260nm)
Time
[min.]

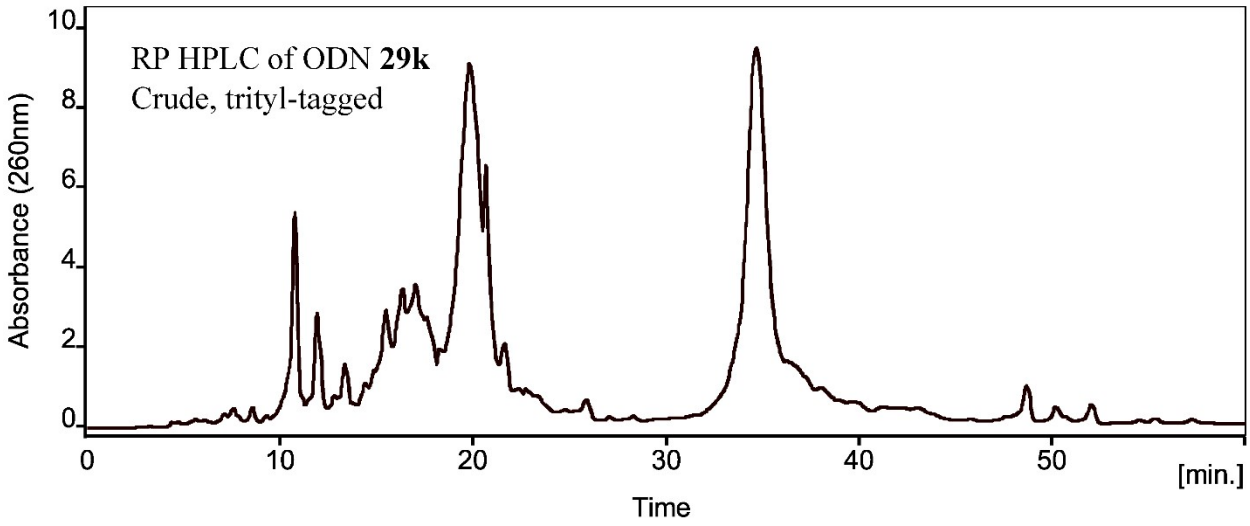

RP HPLC of ODN **29j**
Crude, trityl-tagged
Absorbance (260nm)
0
1
2
3
4
5
6
0
10
20
30
40
50
[min.]
Time

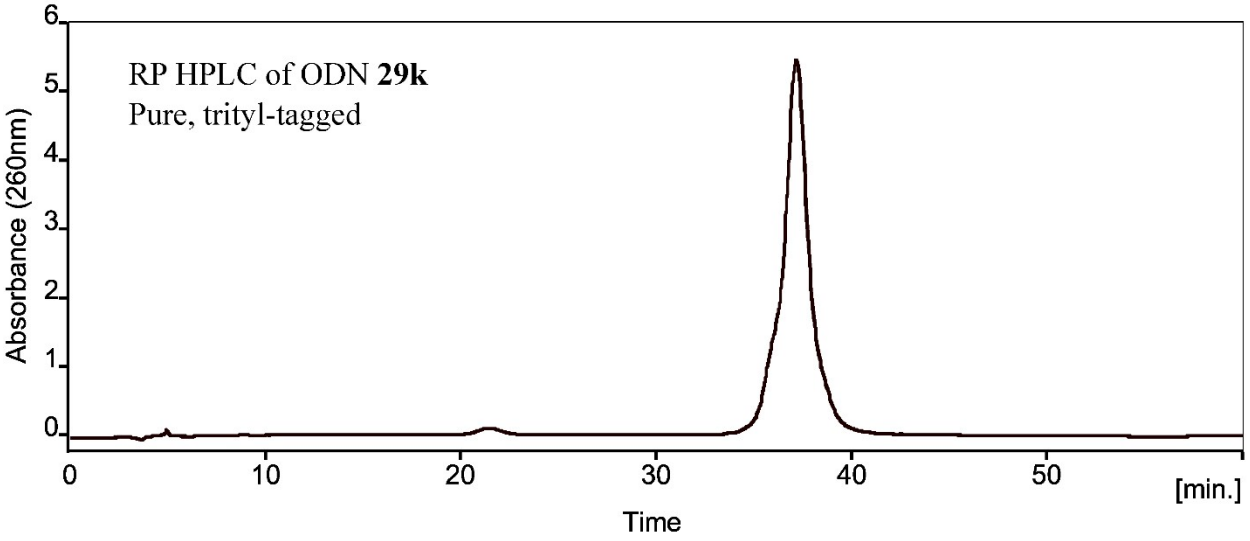

RP HPLC of ODN **29j**
Pure, trityl-tagged
Absorbance (260nm)
0
1
2
3
4
5
0
10
20
30
40
50
[min.]
Time

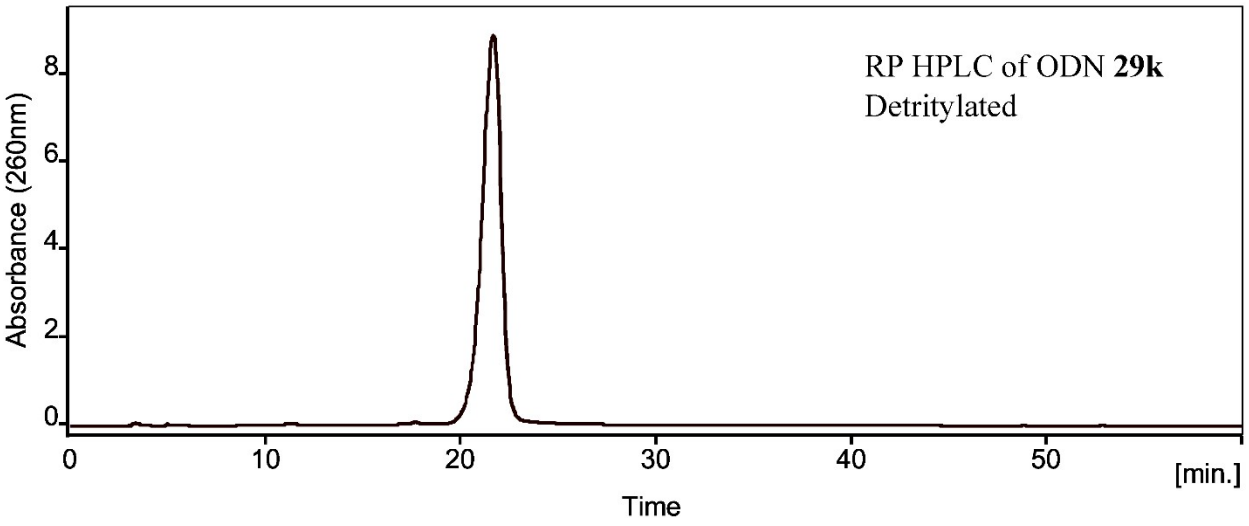

RP HPLC of ODN **29j**
Detritylated
Absorbance (260nm)
Time
[min.]

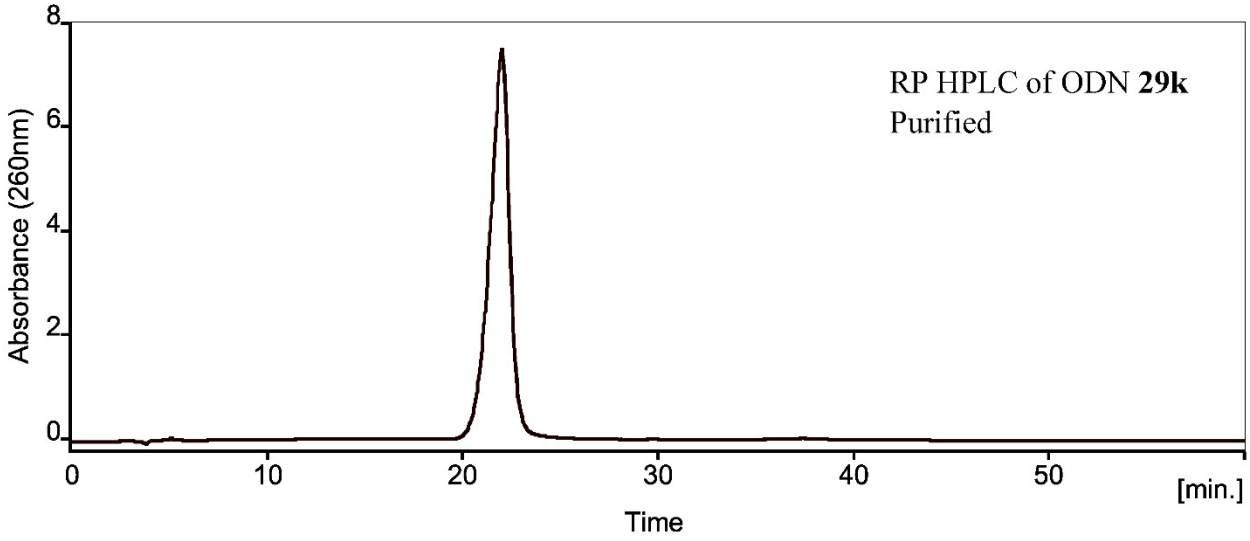

RP HPLC of ODN **29j**
Purified
Absorbance (260nm)
Time
[min.]

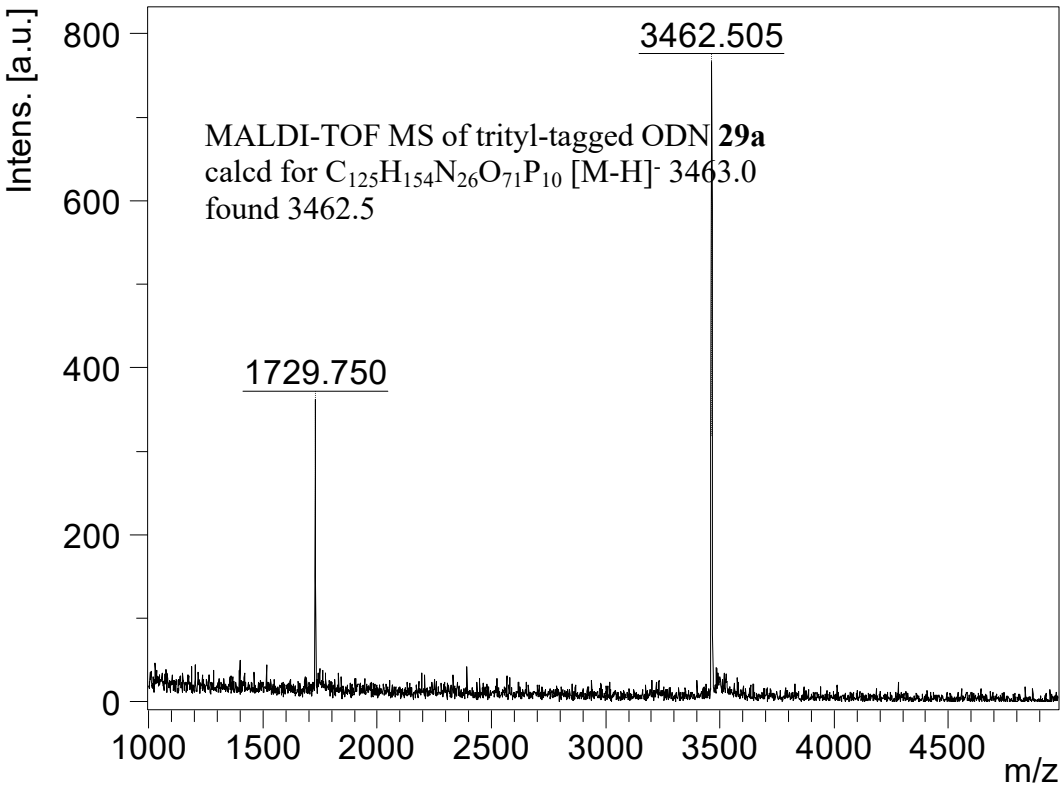

ODN **29j**
RP HPLC of purified ODN **29j** under denatured RP HPLC conditions (see General, Experimental Details in ESI for details)
Materials from buffer
Absorbance (260nm)
Time
[min.]

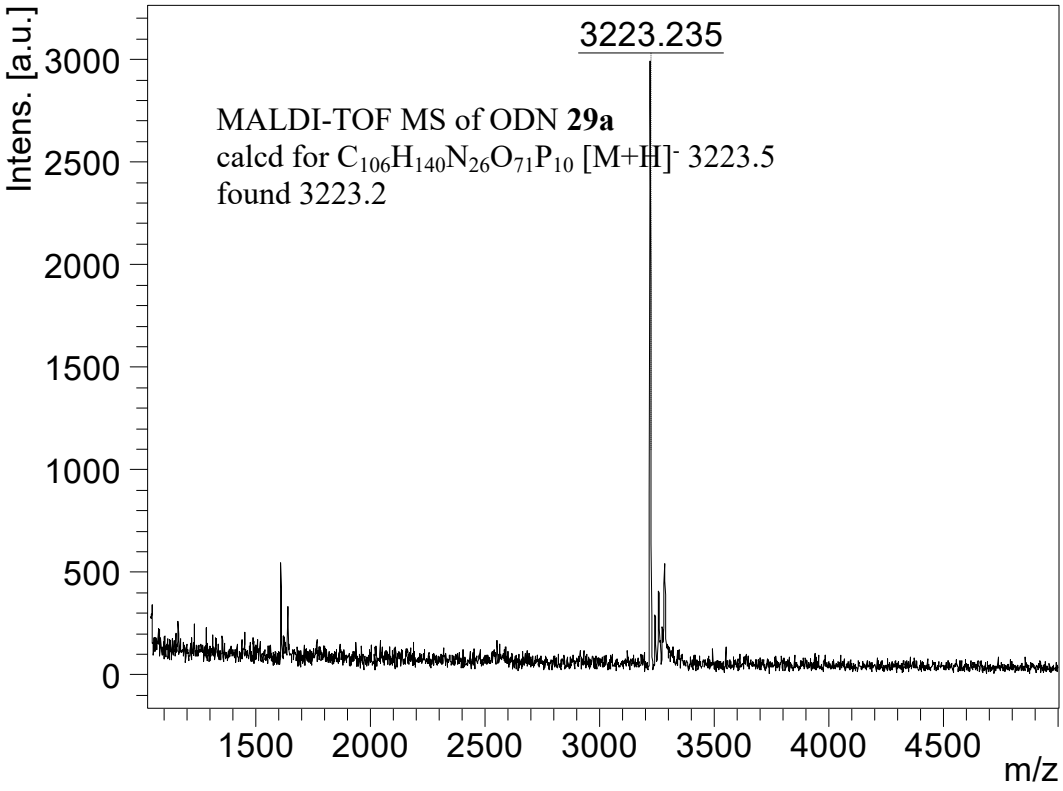

RP HPLC of ODN **29k**
Crude, trityl-tagged
Absorbance (260nm)
10
8
6
4
2
0
0
10
20
30
40
50
[min.]
Time


RP HPLC of ODN **29k**
Pure, trityl-tagged
Absorbance (260nm)
6
5
4
3
2
1
0
0
10
20
30
40
50
[min.]
Time

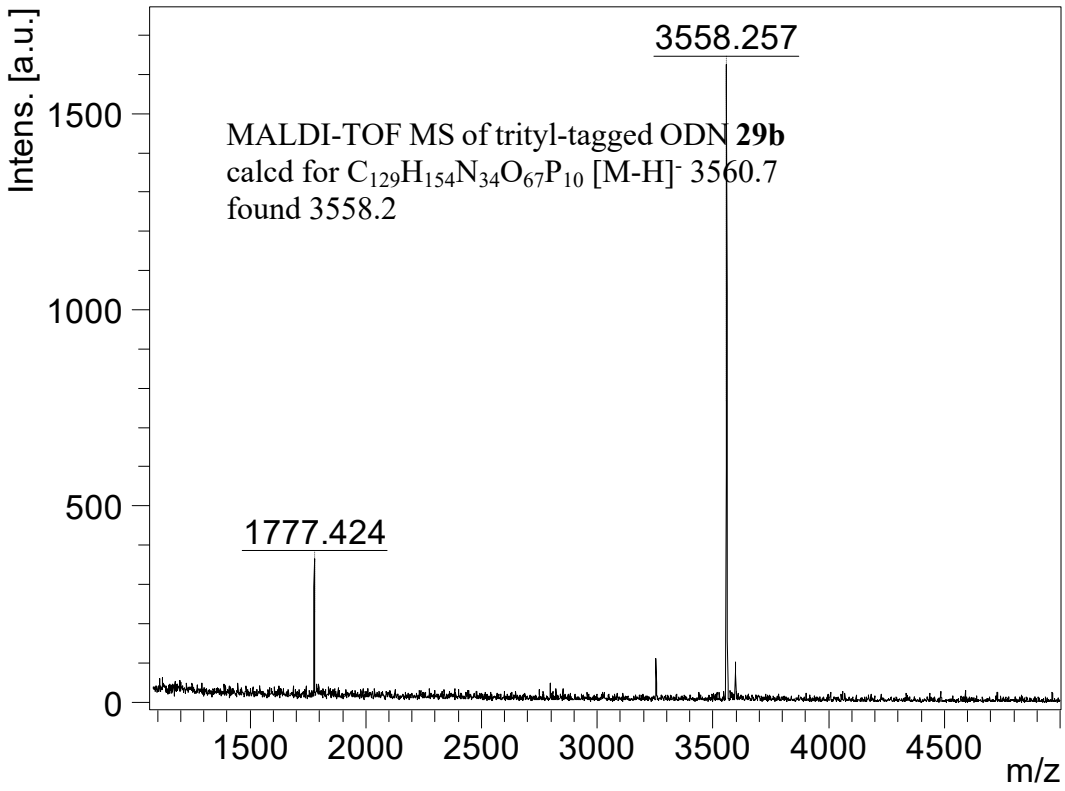

RP HPLC of ODN **29k**
Detritylated
Absorbance (260nm)
8
6
4
2
0
0
10
20
30
40
50
[min.]
Time

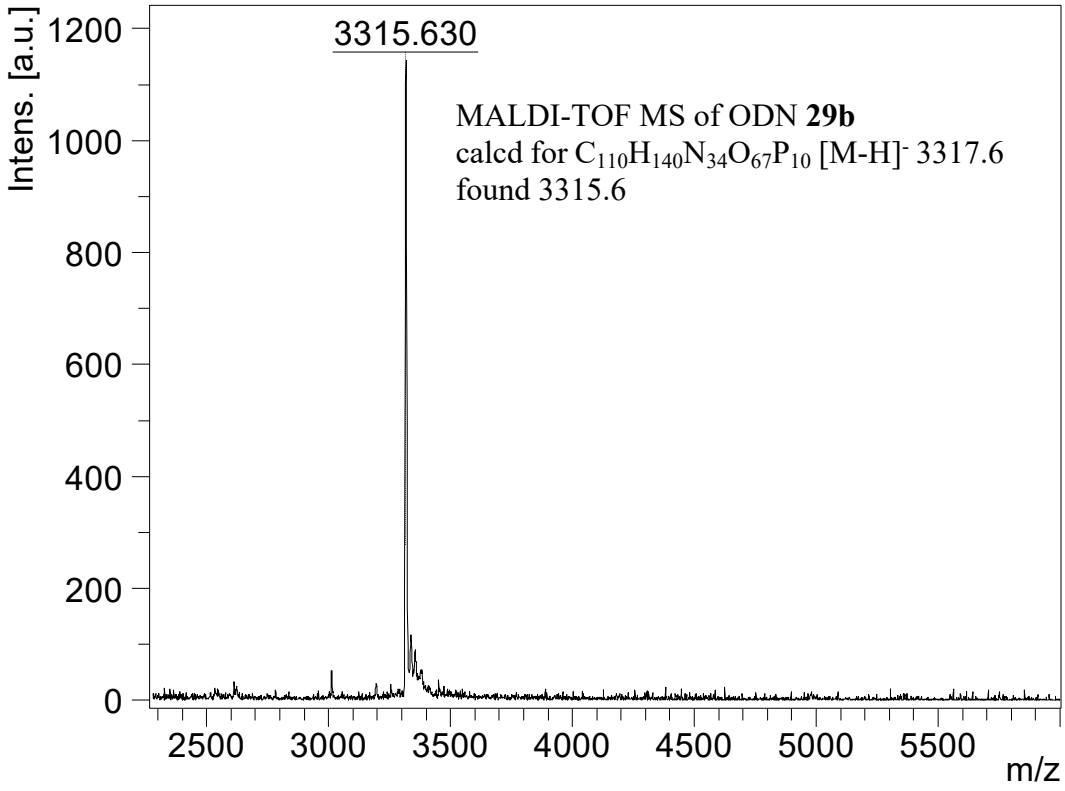

RP HPLC of ODN **29k**
Purified
Absorbance (260nm)
8
6
4
2
0
0
10
20
30
40
50
[min.]
Time



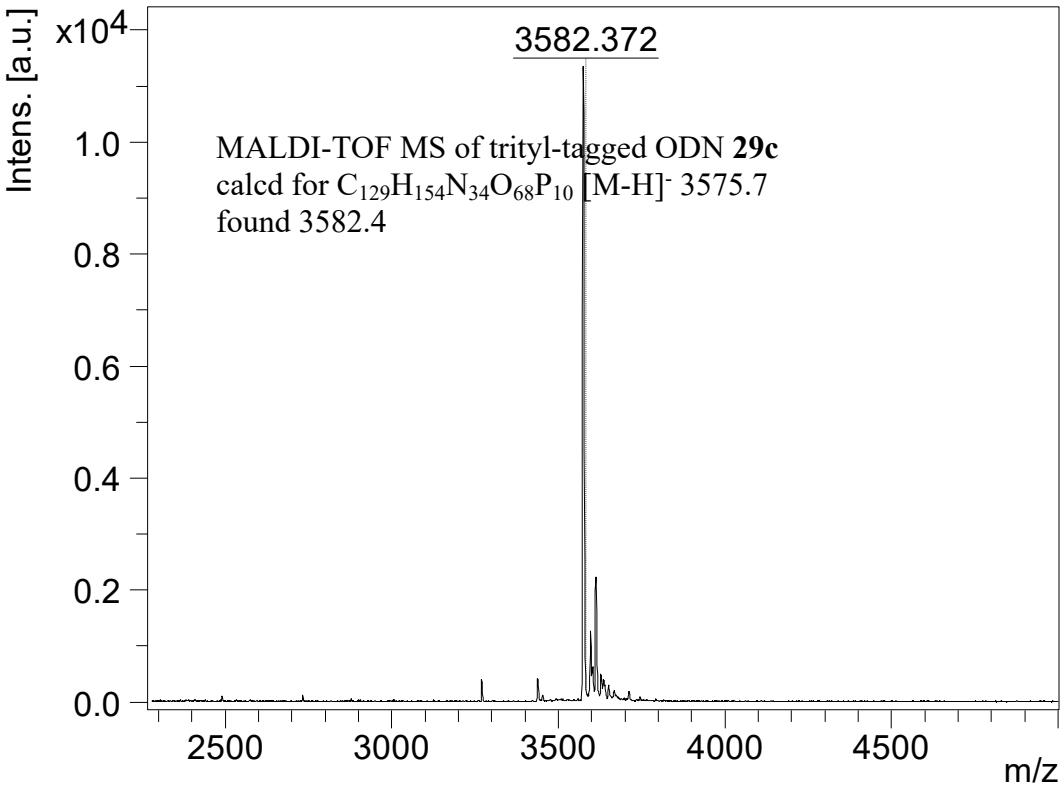


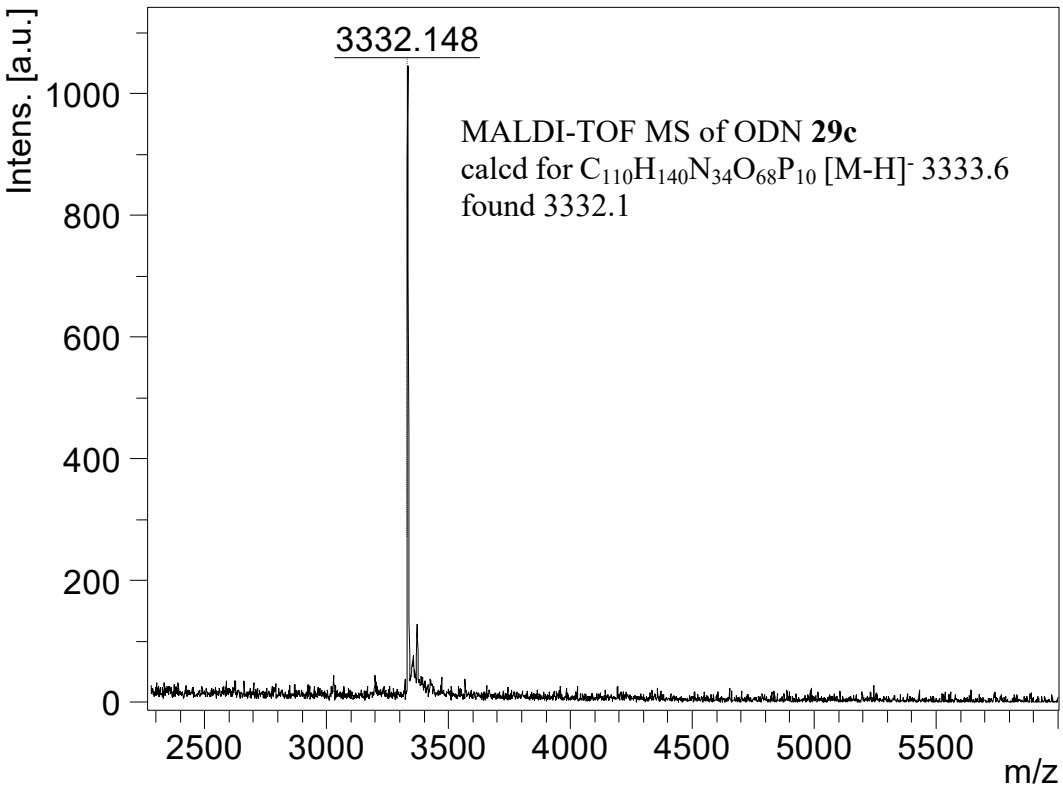
OD_{260} of ODN **29a** for the 0.52 μmol synthesis is 2.940.



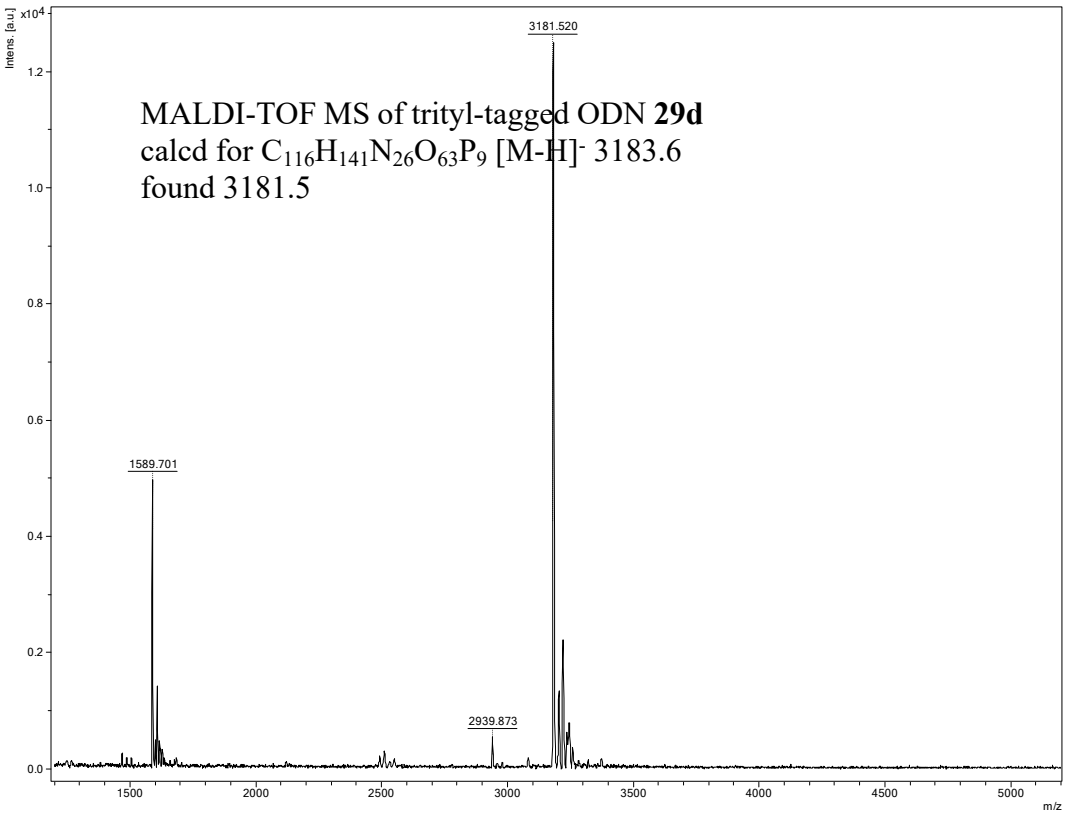


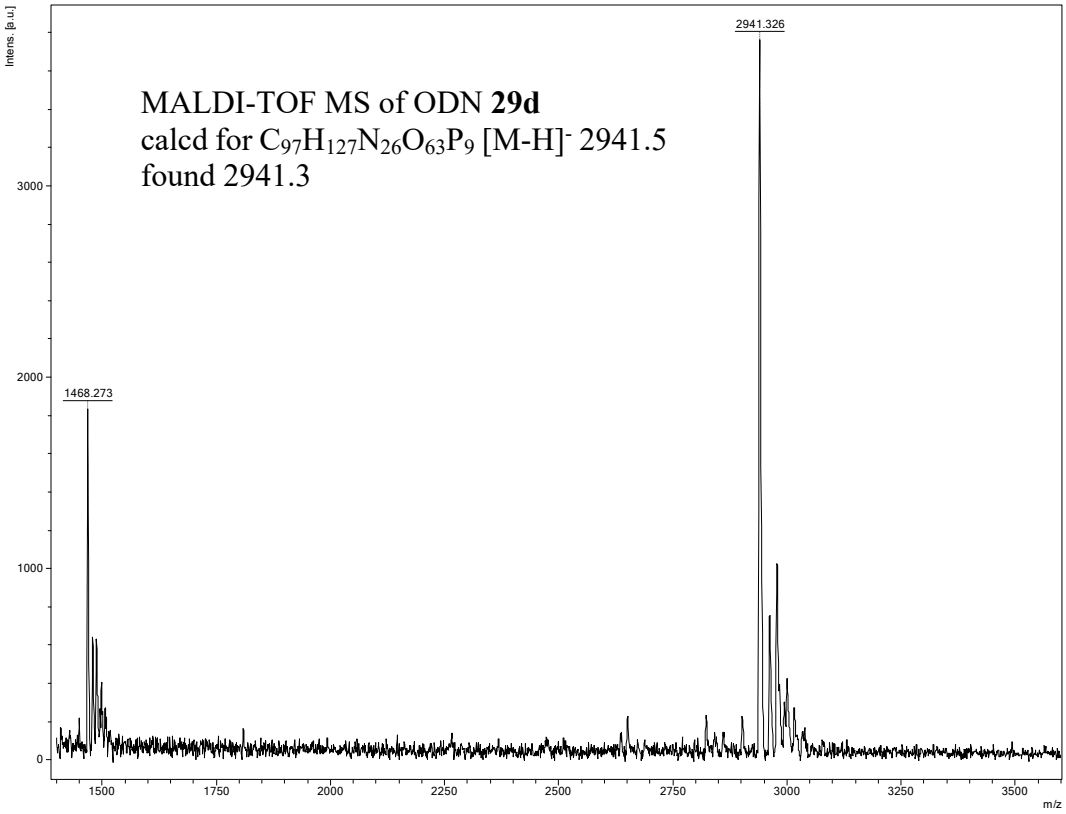
OD_{260} of ODN **29b** for the 0.52 μmol synthesis is 3.660.



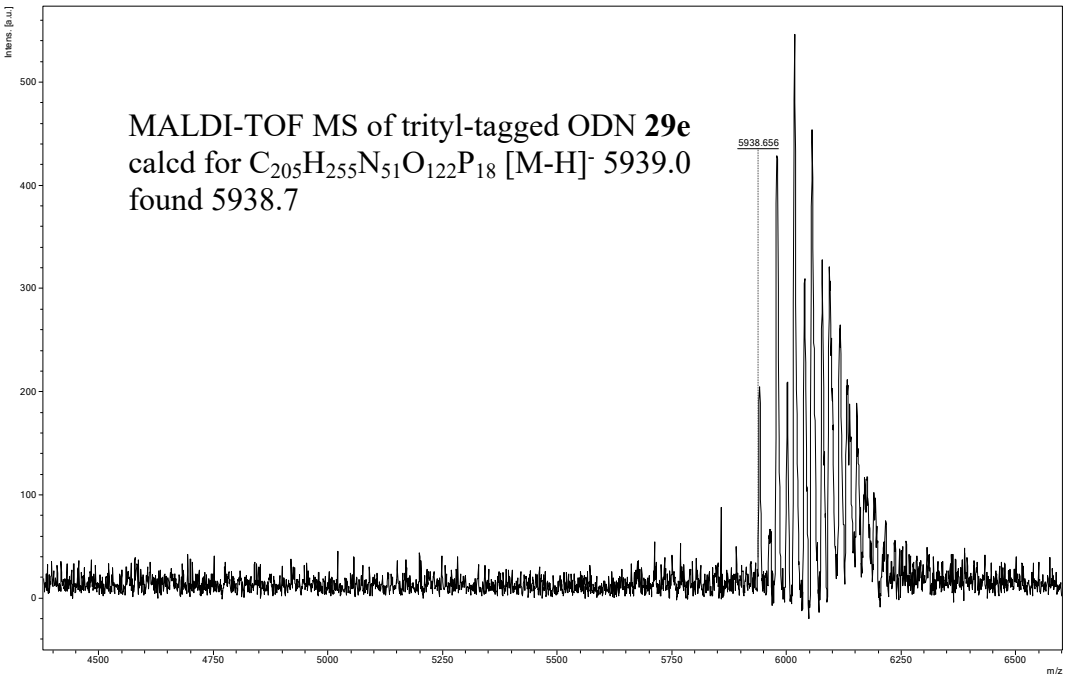


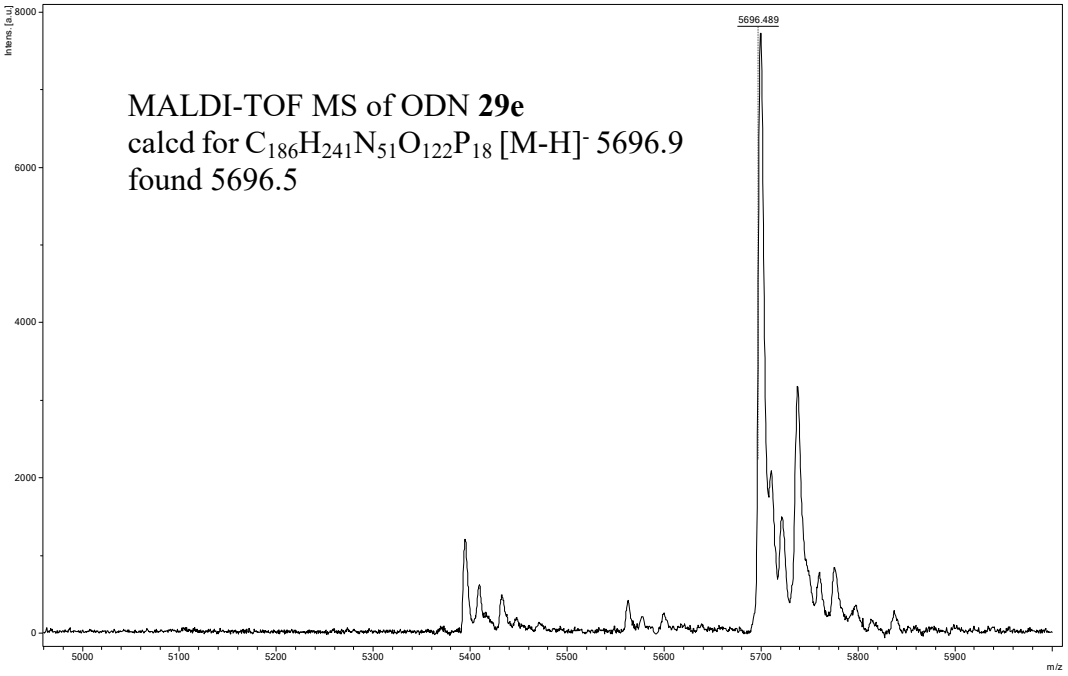
OD_{260} of ODN **29c** for the 0.52 μmol synthesis is 3.092.



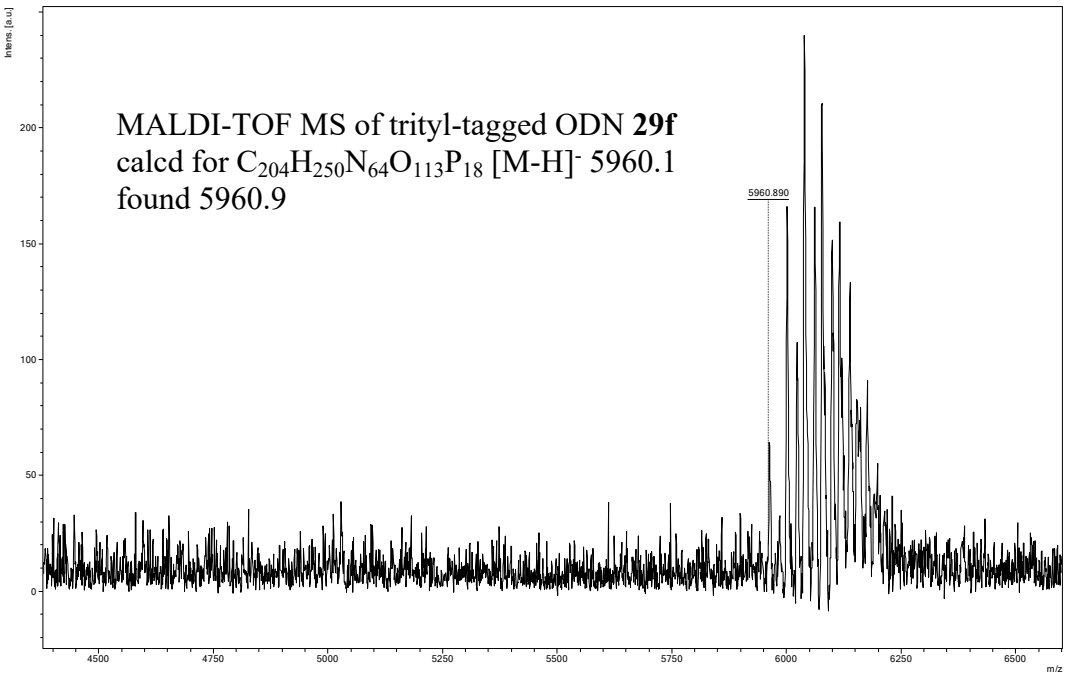


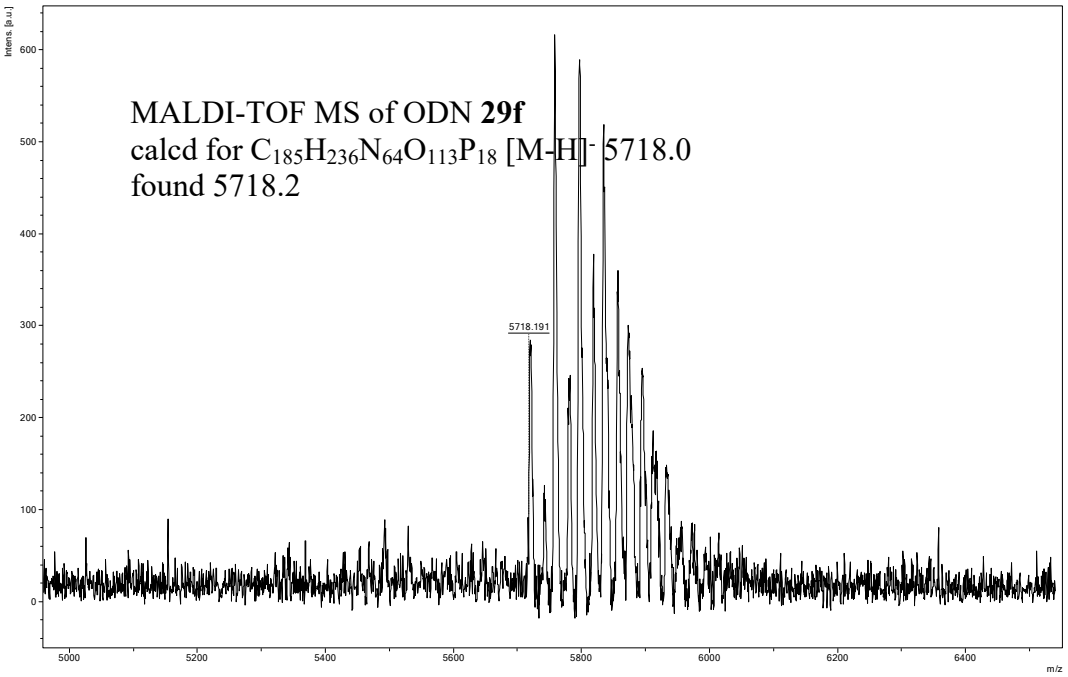
OD_{260} of ODN **29d** for the 0.52 µmol synthesis is 0.612.



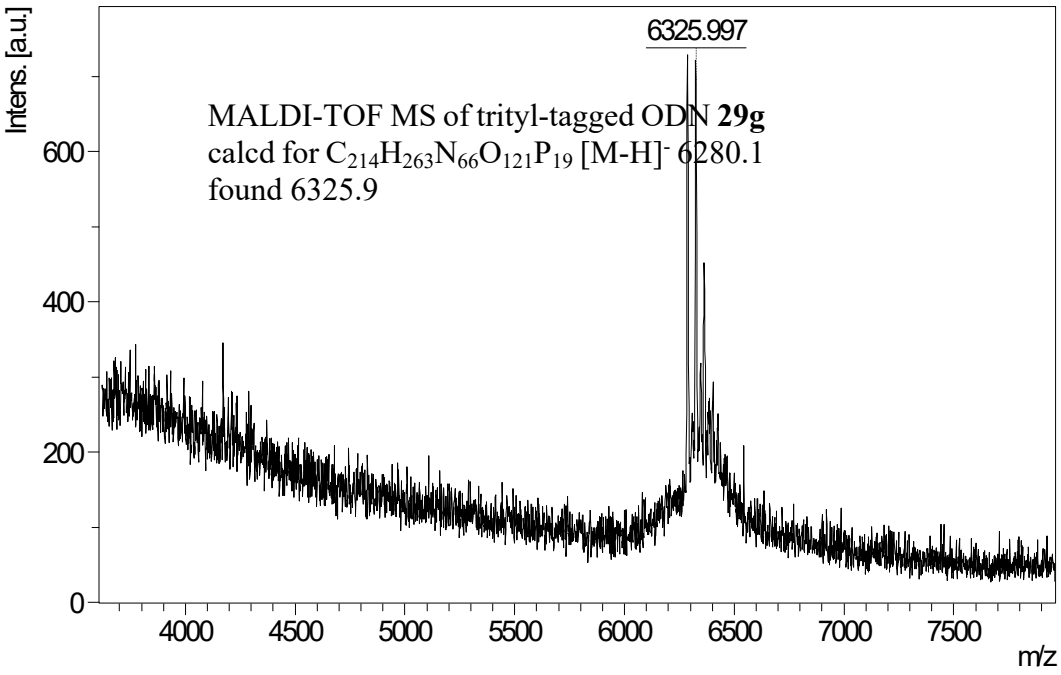


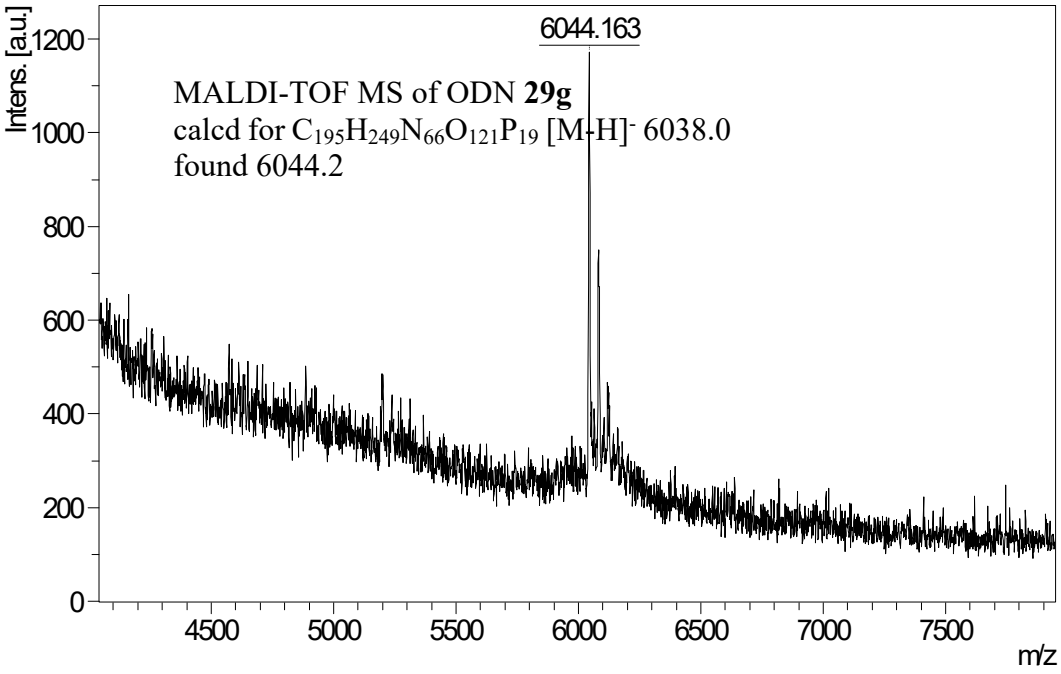
OD_{260} of ODN **29e** for the 0.52 μmol synthesis is 0.542.



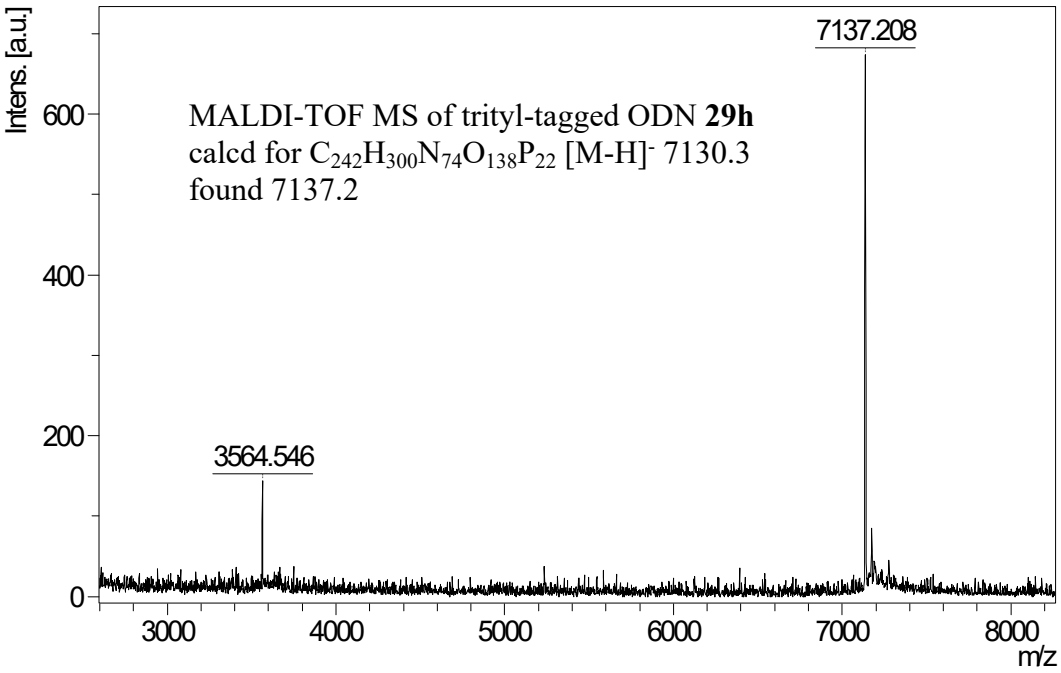


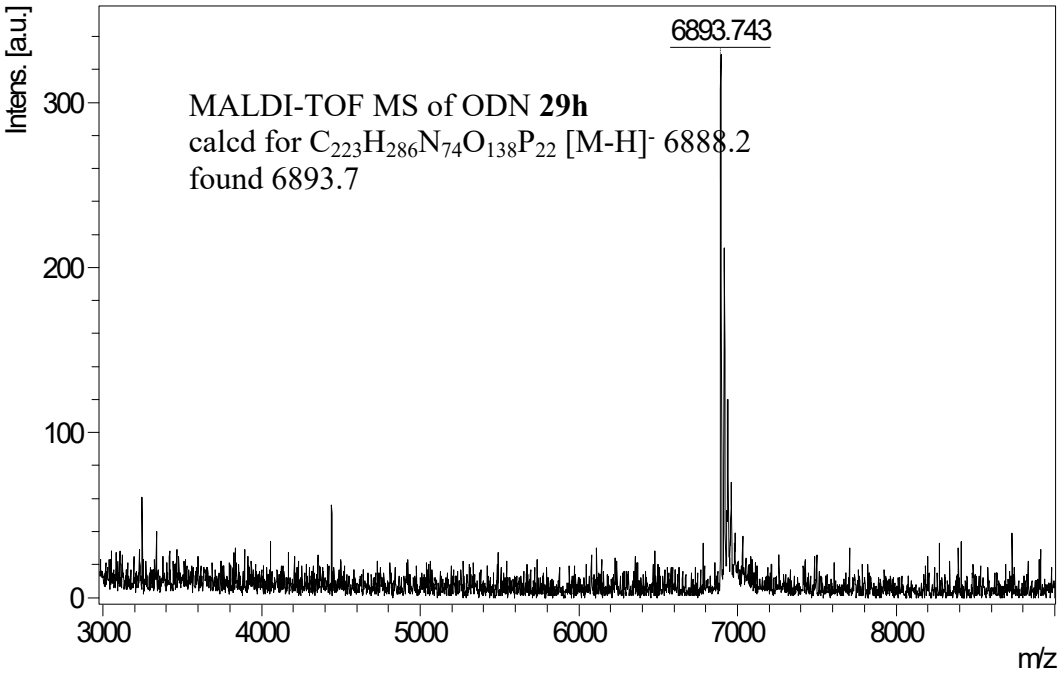
OD_{260} of ODN **29f** for the 0.52 μmol synthesis is 1.119.



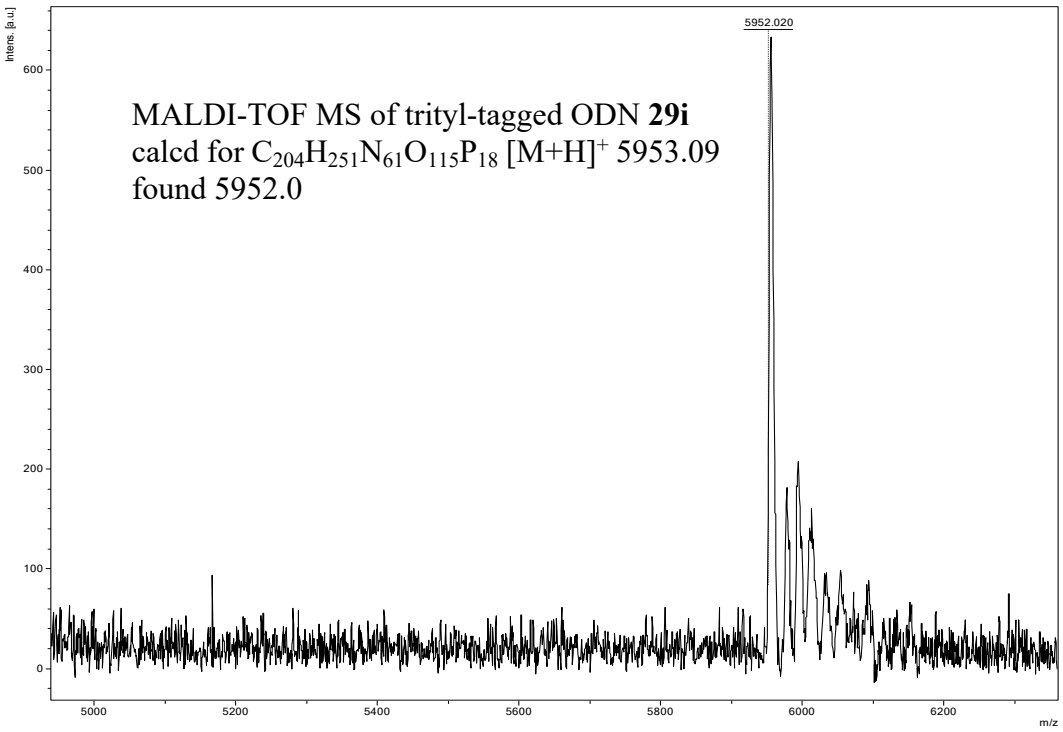


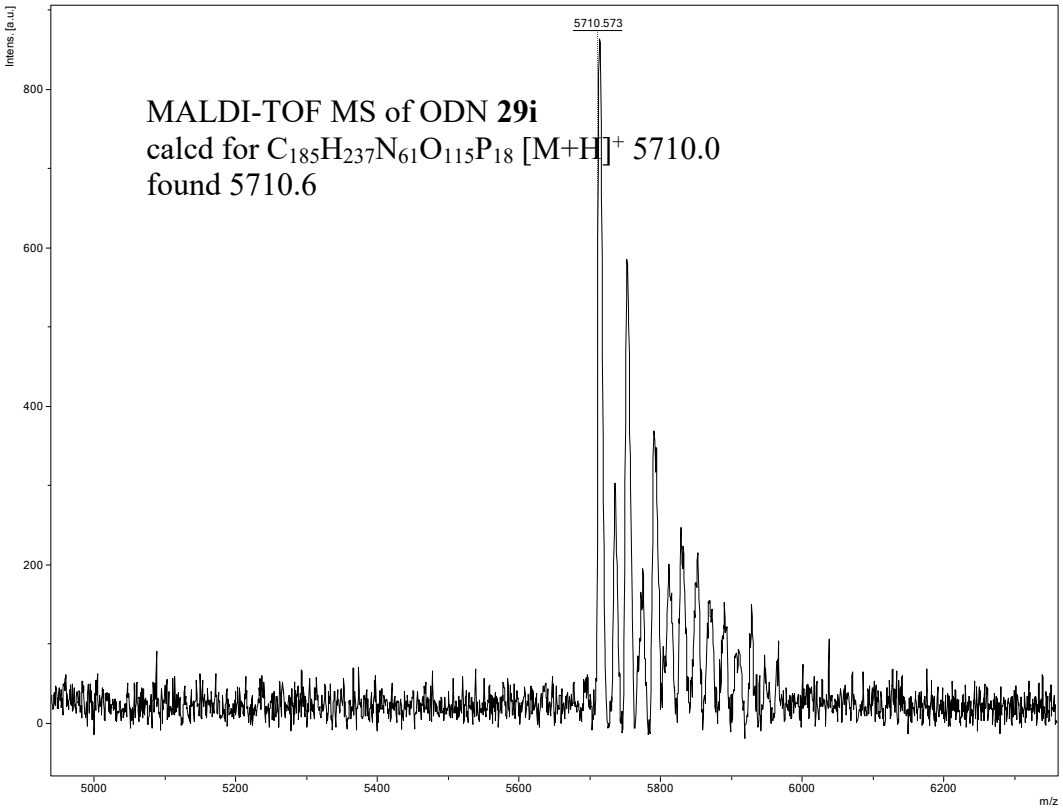
OD_{260} of ODN **29g** for the 0.52 μmol synthesis is 1.144.



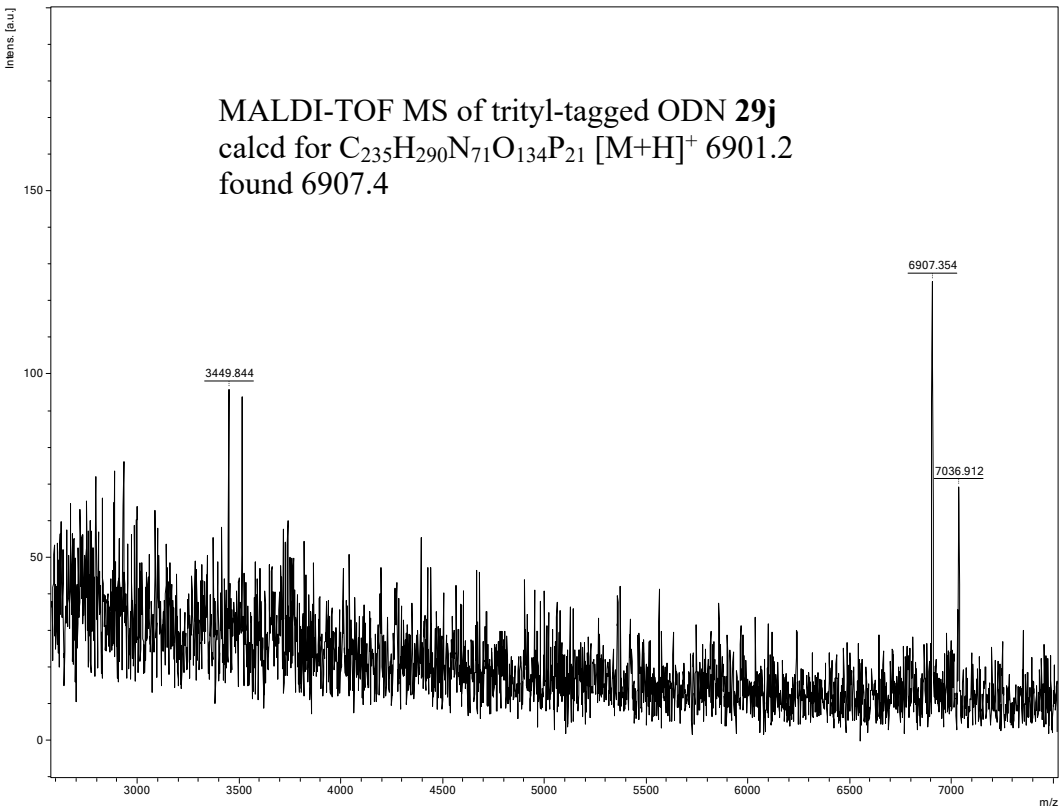


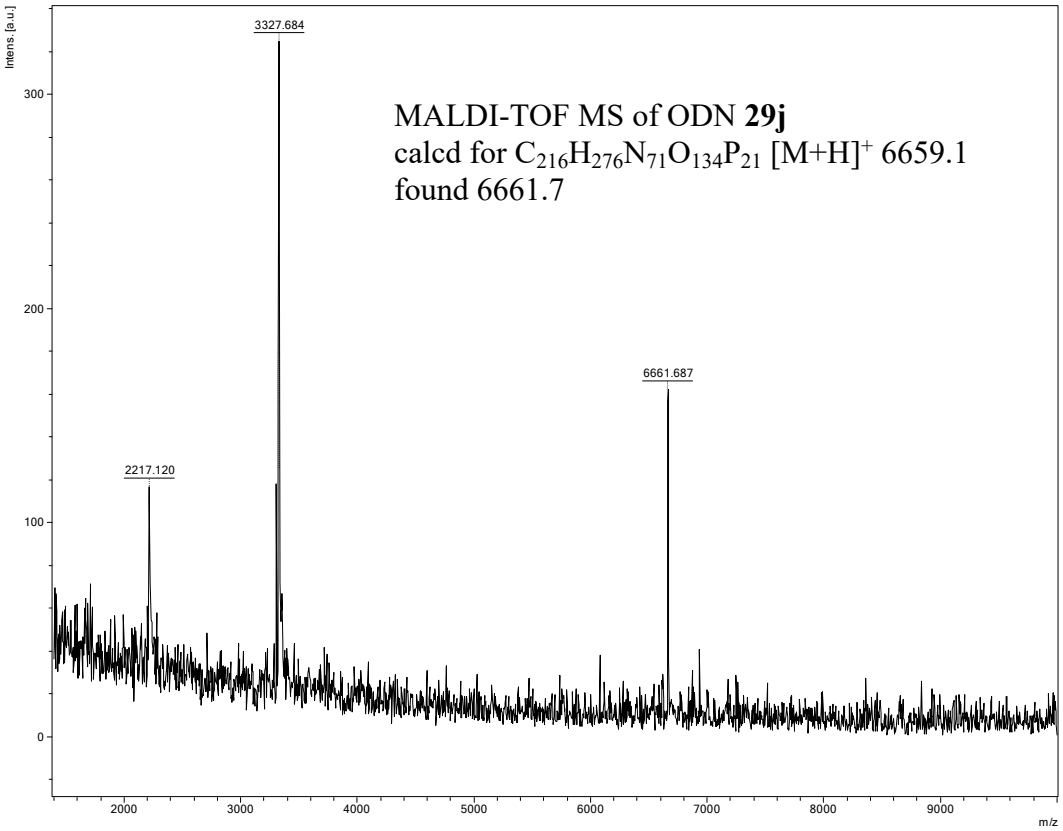
OD_{260} of ODN **29h** for the 0.52 μmol synthesis is 3.243.



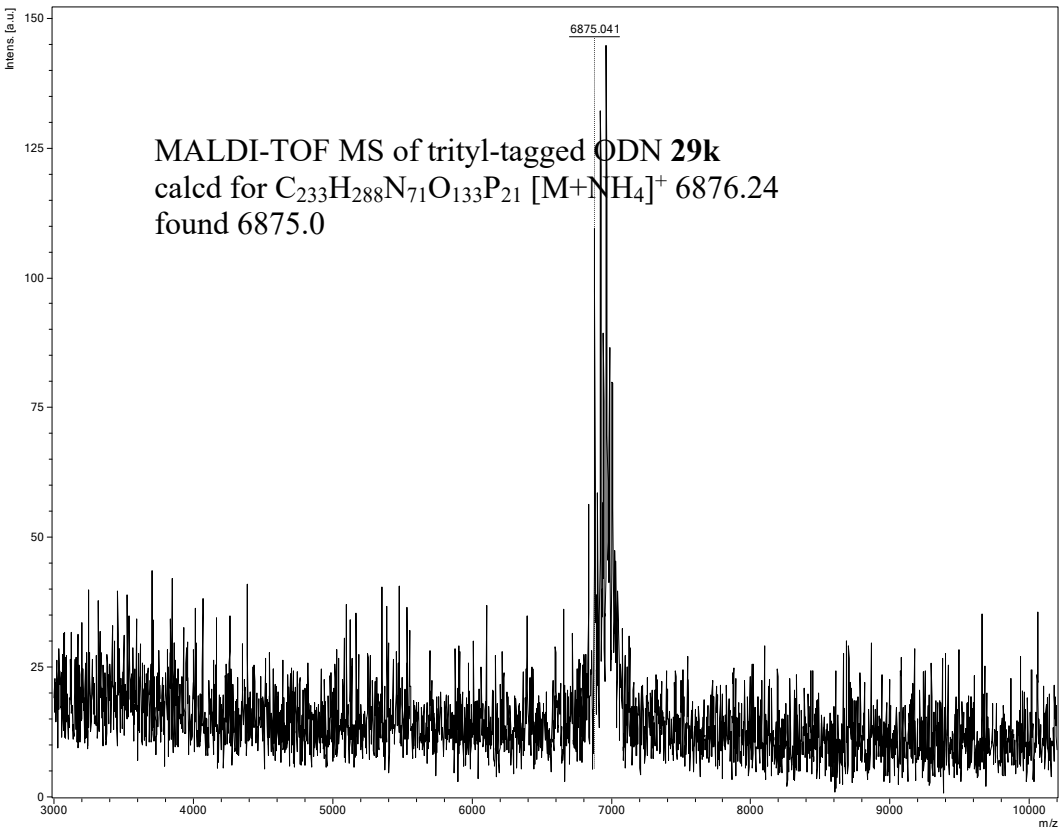


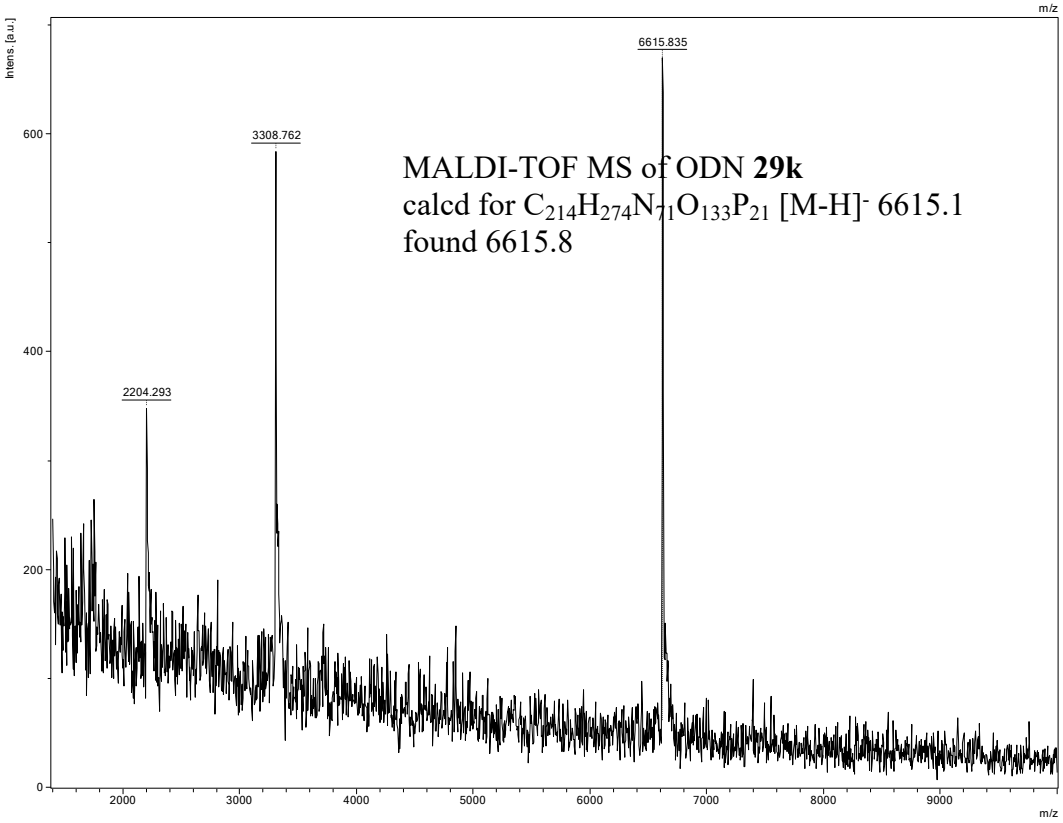
OD_{260} of ODN **29i** for the 0.52 μmol synthesis is 1.461.



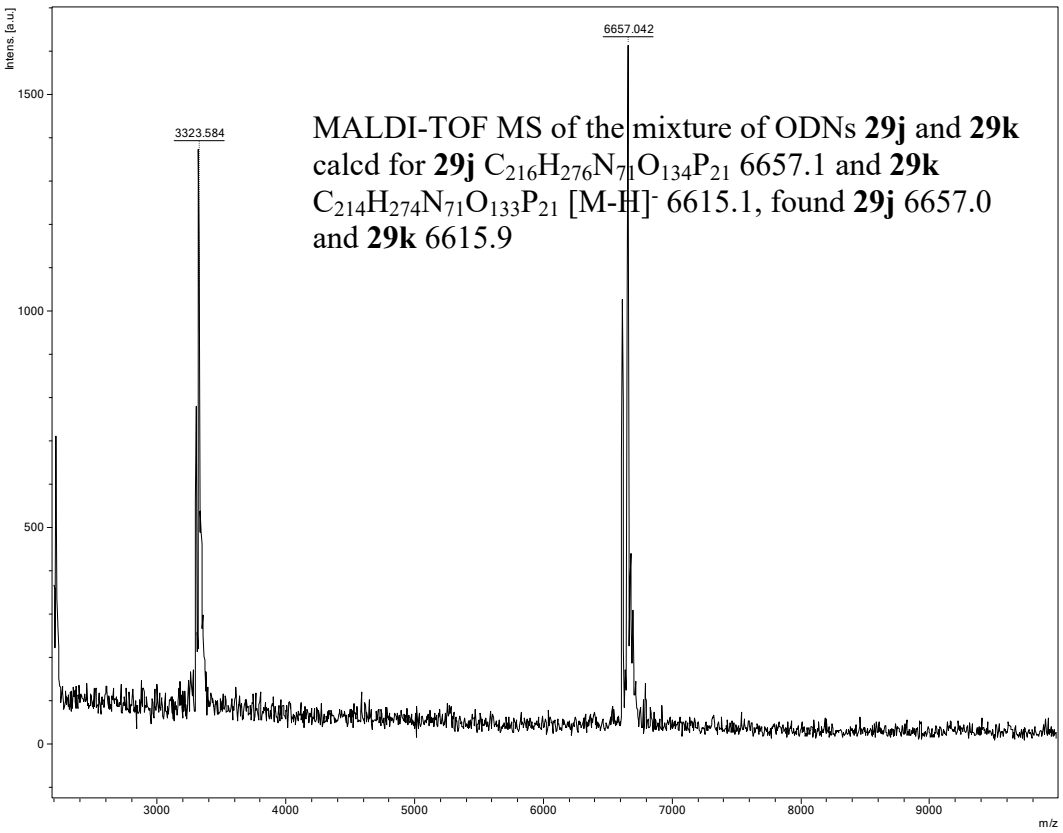


OD_{260} of ODN **29j** for the 0.52 µmol synthesis is 0.536.

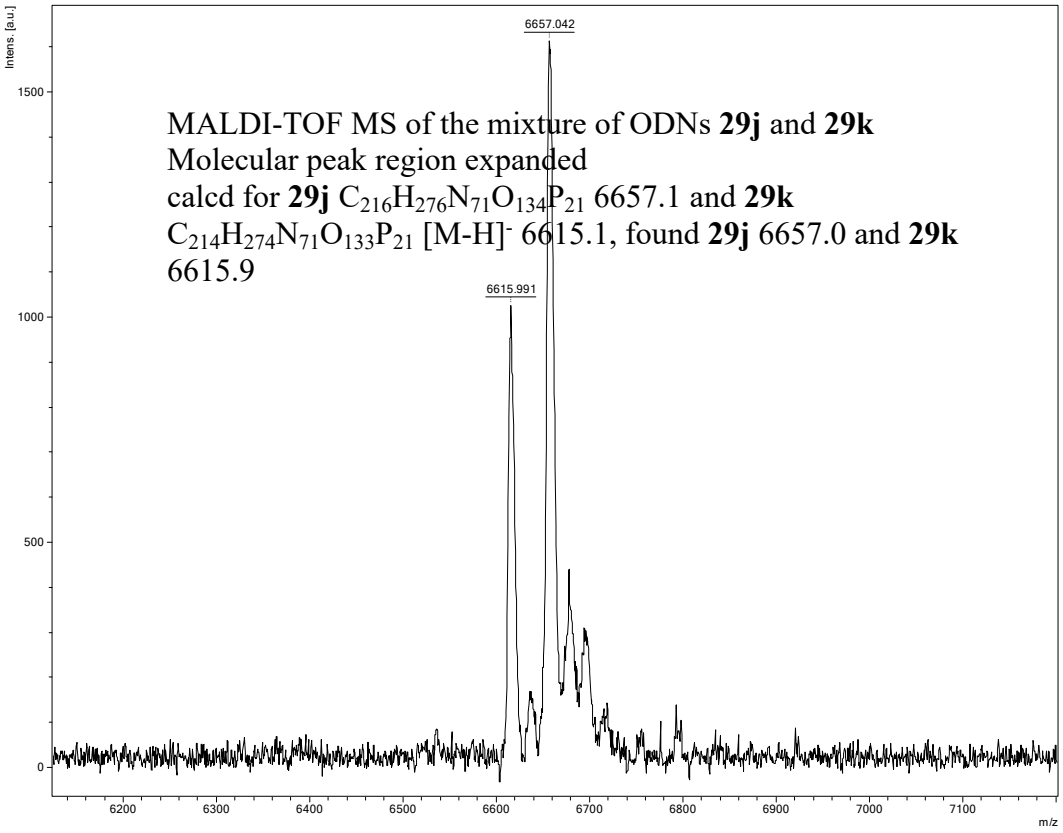




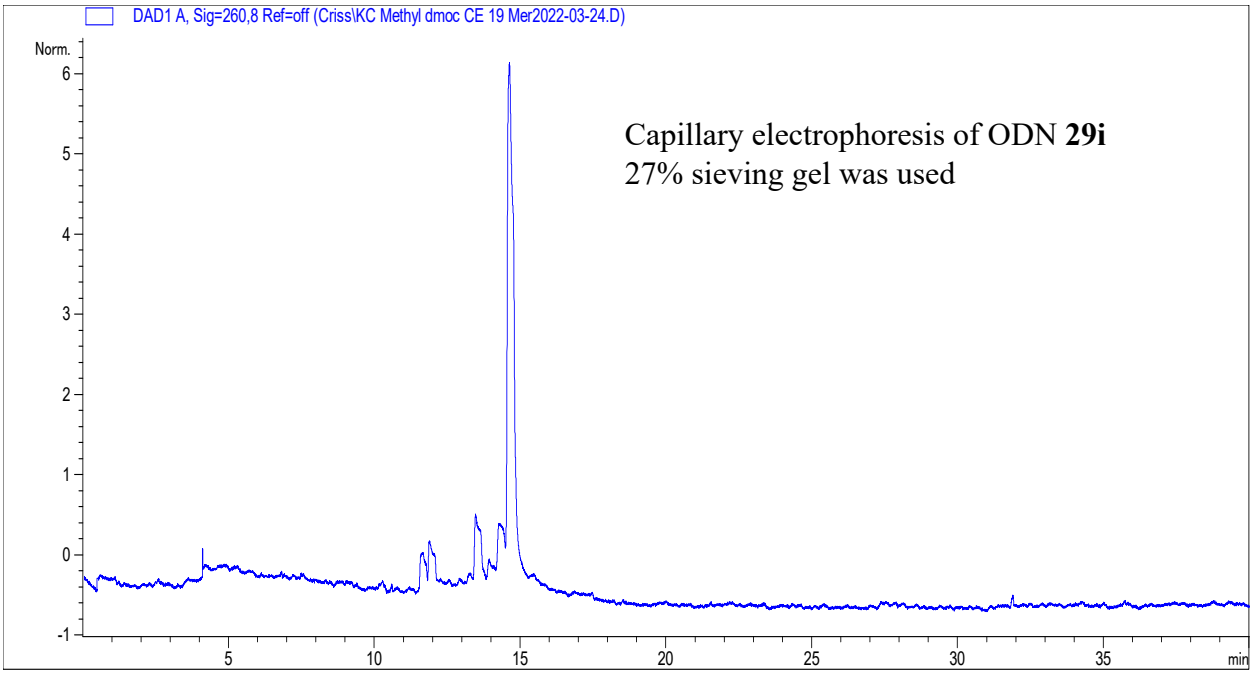
OD_{260} of ODN **29k** for the 0.52 μmol synthesis is 2.717.



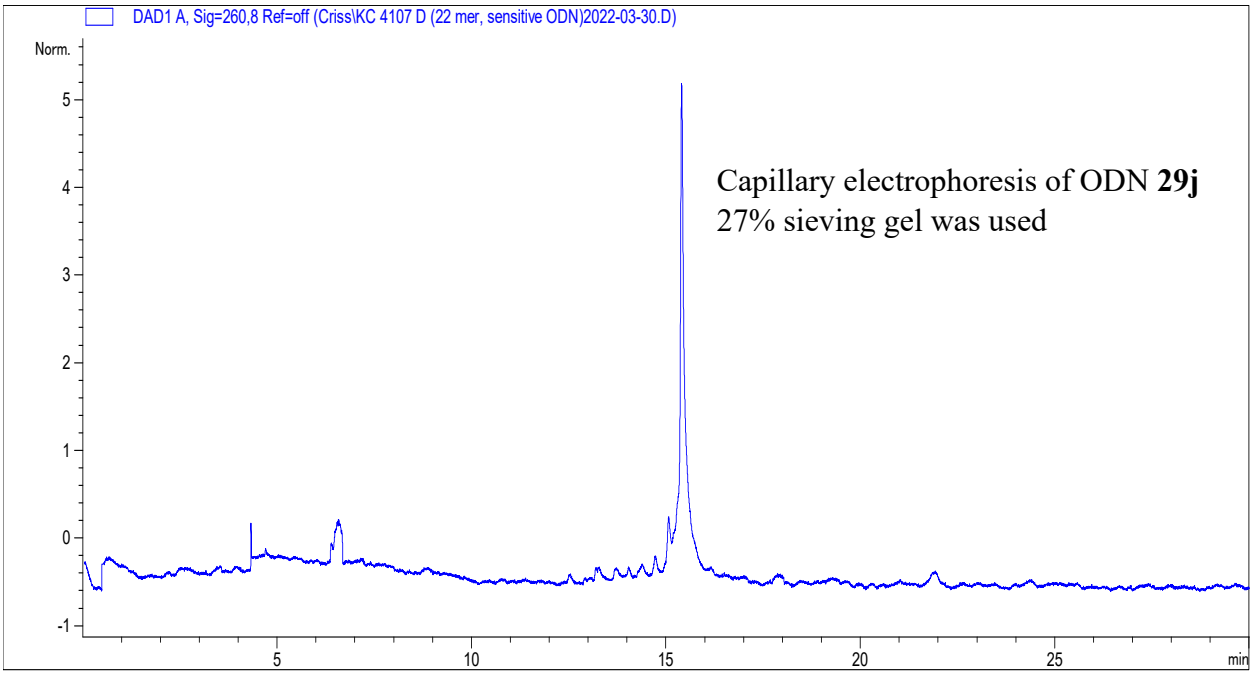
MALDI-TOF MS of the mixture of ODNs **29j** and **29k**
calcd for **29j** $C_{216}H_{276}N_{71}O_{134}P_{21}$ 6657.1 and **29k** $C_{214}H_{274}N_{71}O_{133}P_{21}$ $[M-H]^-$ 6615.1, found **29j** 6657.0 and **29k** 6615.9



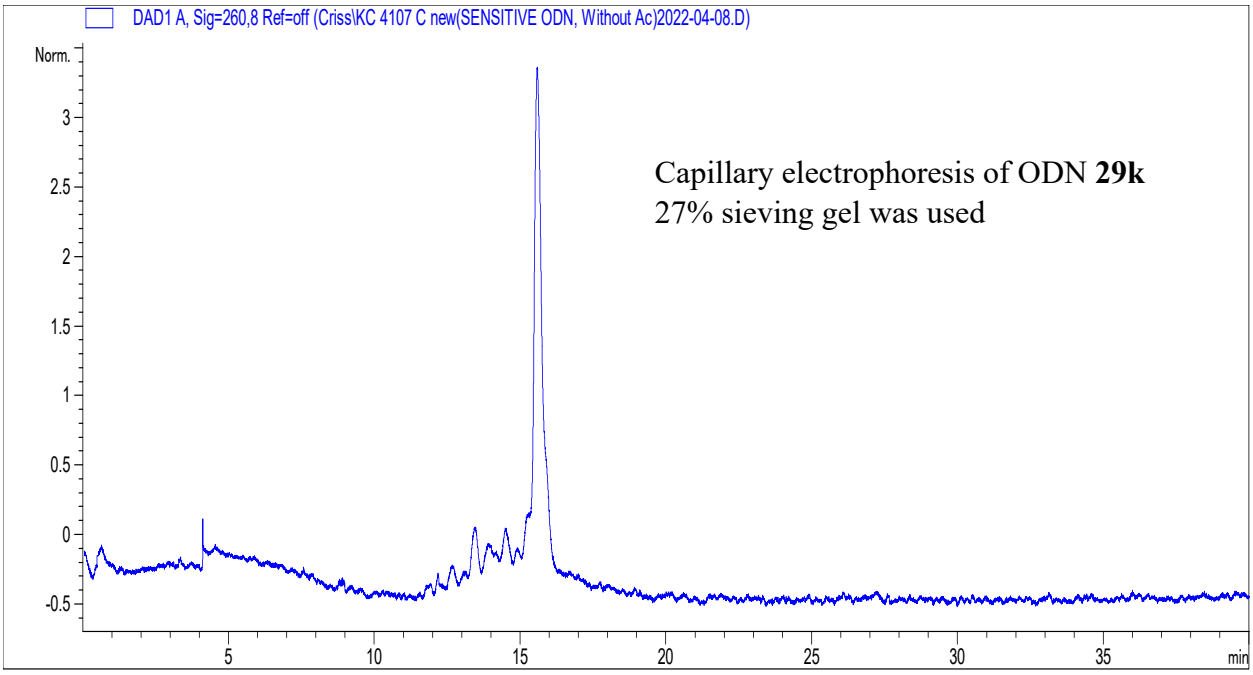
MALDI-TOF MS of the mixture of ODNs **29j** and **29k**
Molecular peak region expanded
calcd for **29j** $C_{216}H_{276}N_{71}O_{134}P_{21}$ 6657.1 and **29k** $C_{214}H_{274}N_{71}O_{133}P_{21}$ $[M-H]^-$ 6615.1, found **29j** 6657.0 and **29k** 6615.9



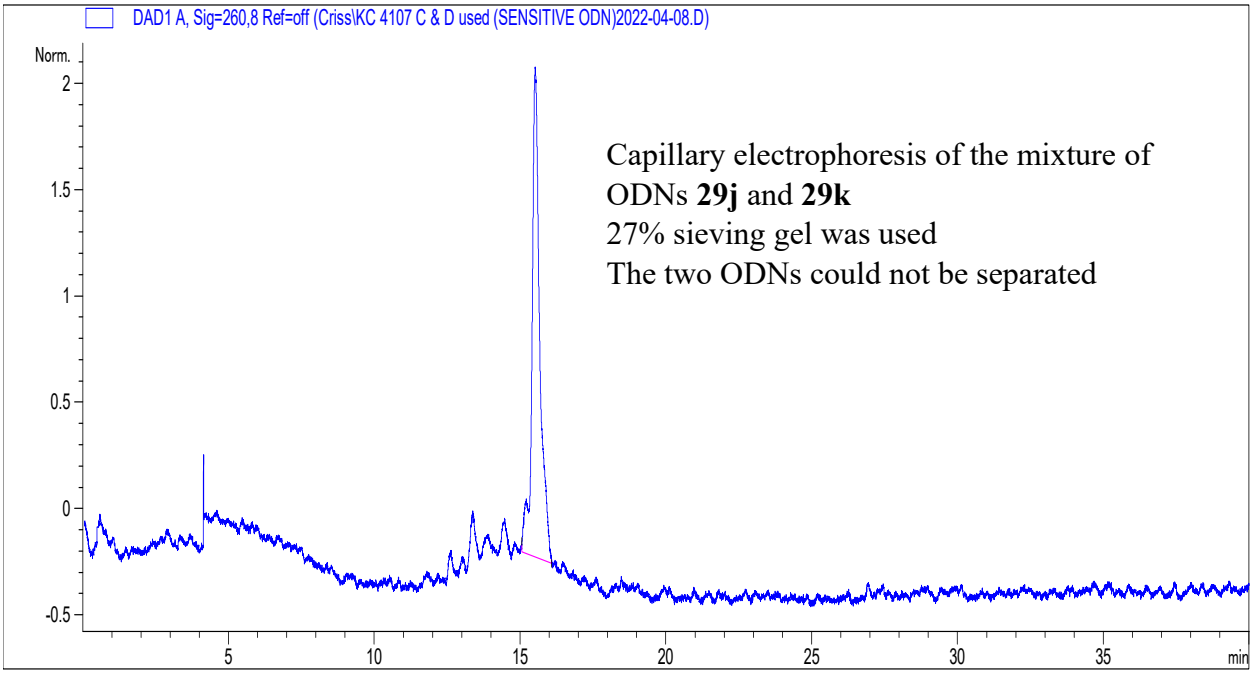
Capillary electrophoresis of ODN **29i**
27% sieving gel was used



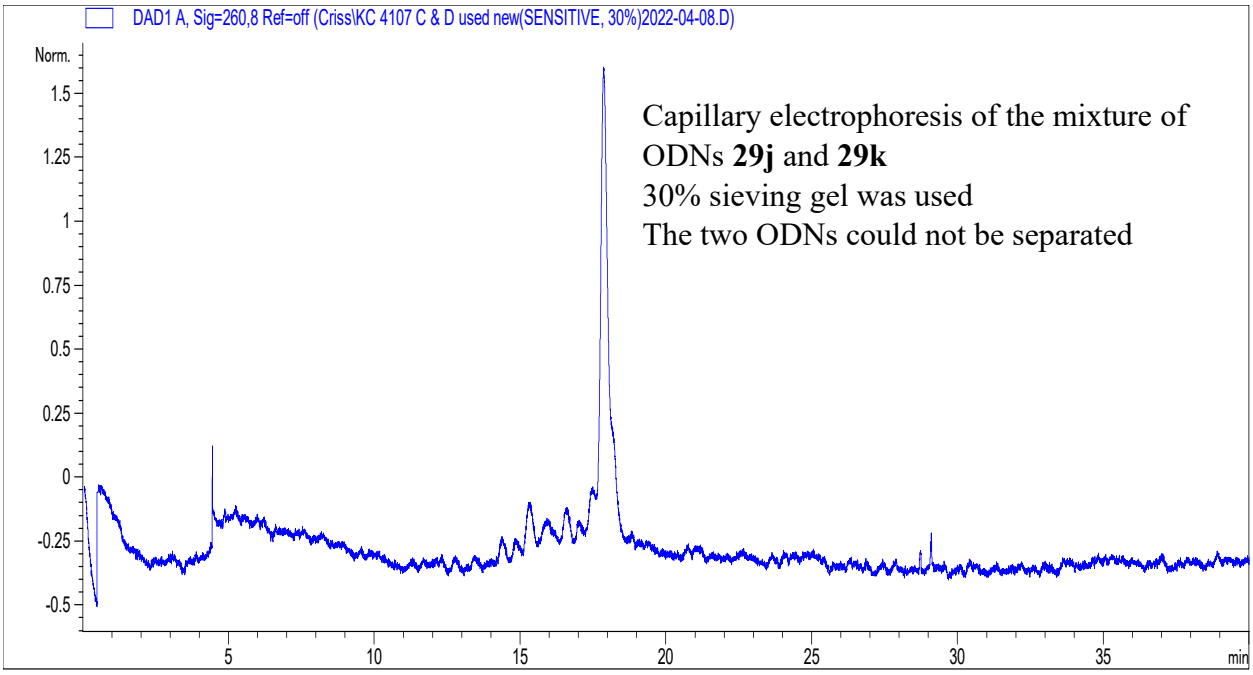
Capillary electrophoresis of ODN **29j**
27% sieving gel was used



Capillary electrophoresis of ODN **29k**
27% sieving gel was used



Capillary electrophoresis of the mixture of ODNs **29j** and **29k**
27% sieving gel was used
The two ODNs could not be separated

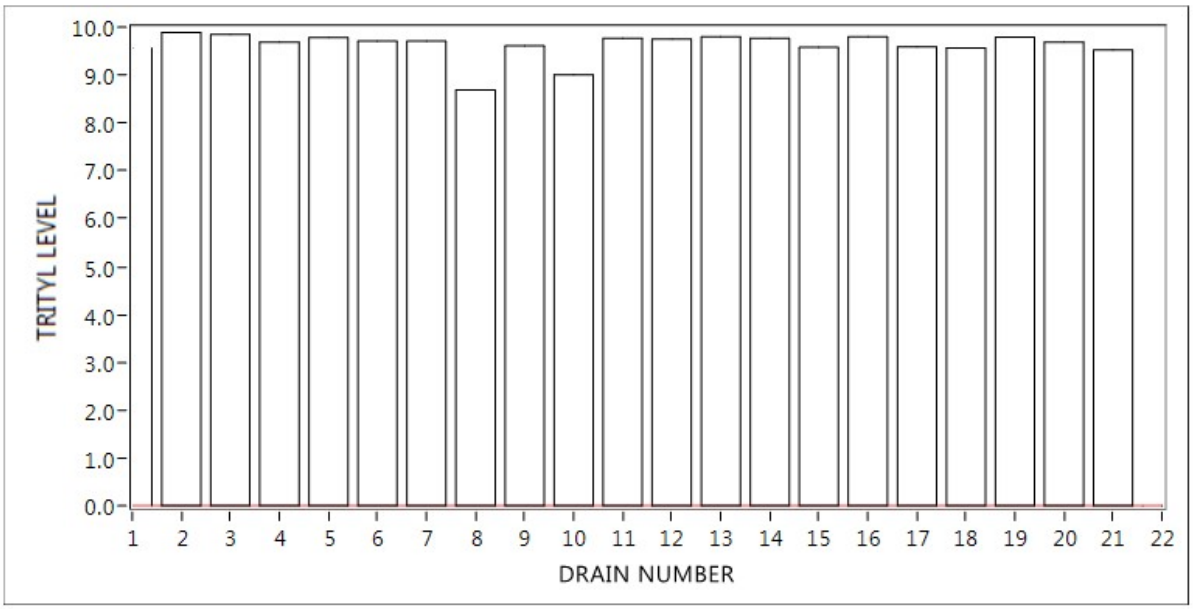

DAD1 A, Sig=260,8 Ref=off (Criss\KC 4107 C & D used new(SENSITIVE, 30%)2022-04-08.D)
Norm.
1.5
1.25
1
0.75
0.5
0.25
0
-0.25
-0.5
5
10
15
20
25
30
35
min
Capillary electrophoresis of the mixture of ODNs **29j** and **29k**
30% sieving gel was used
The two ODNs could not be separated

An example trityl assay log of ODN synthesis

RUN NAME: KC 4107 (22 mer, Sensitive MD CE

RUN DATE: 20220310 21:36:27

POSITION: COLUMN 1



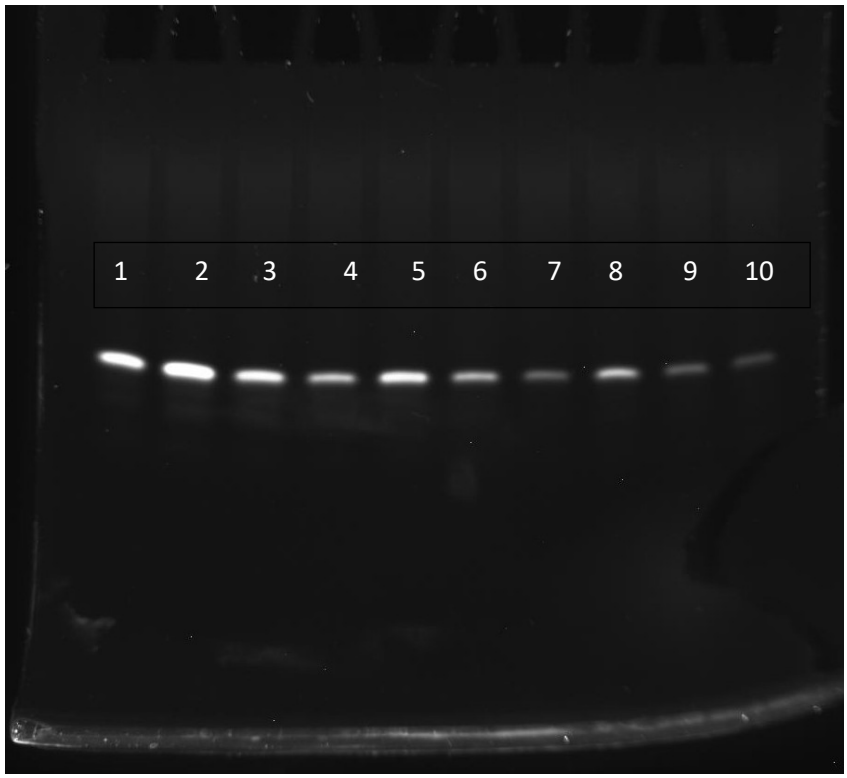


Image of denatured polyacrylamide gel electrophoresis analysis of ODN **29j** in comparison with **29k**. The two ODNs have the same sequence but **29j** has a 4acC modification while **29k** does not. Polyacrylamide gel (15%, 7 M urea), 1X TBE buffer, 190 V, 45 min, stained with Gel Red.

Lane 1: 48 ng **29j**. Lane 2: 48 ng **29j** and 46.5 ng **29k**. Lane 3: 46.5 ng **29k**.

Lane 4: 24 ng **29j**. Lane 5: 24 ng **29j** and 23.3 ng **29k**. Lane 6: 23.3 ng **29k**.

Lane 7: 12 ng **29j**. Lane 8: 12 ng **29j** and 11.6 ng **29k**. Lane 9: 11.6 ng **29k**.

Lane 10: 7.2 ng **29j**

Although the two ODNs could not be resolved, the single bands indicate that they do not contain any sequences that are shorter or longer than them. In addition, as presented in other parts of the paper, MALDI MS indicates that **29j** contains the 4acC modification while **29k** does not (Figure 4). Considering these and other evidences, which include CE and HPLC analyses, presented in the paper, **29j** is pure.