Parallel, Large Scale and Long Synthetic Oligodeoxynucleotide Purification Using the Catching Full-Length Sequence by Polymerization Technique

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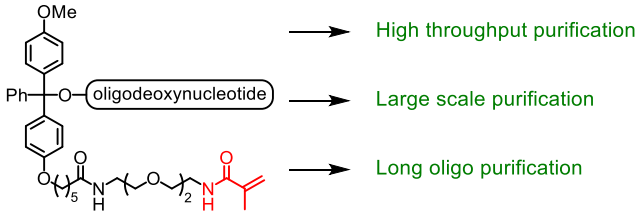


ABSTRACT: The catching by polymerization synthetic oligodeoxynucleotide (ODN) purification technique was shown to be potentially suitable for high throughput purification by purifying 12 ODNs simultaneously, to be convenient for large-scale purification by purifying at 60 μmol synthesis scale, and to be highly powerful for long ODN purification by purifying ODNs as long as 303-mer. LC-MS analysis indicated that the ODNs purified with the technique have excellent purity.

Keyword: oligonucleotide, purification, synthesis, polymerization, large scale, high throughput

INTRODUCTION

In recent years, several emerging research areas including synthetic biology,[1-5] CRISPR genome editing,[6-10] antisense therapeutics[11-12] and DNA data storage[13-16] created a high demand for synthetic oligodeoxynucleotides (ODNs). To meet this demand, high throughput and large-scale ODN production are required. Although significant progress has been made on high throughput and large scale ODN synthesis in the last few years, limited progress has been made on high throughput and large-scale purification.[17-23] Currently ODN purification technologies mainly include HPLC and gel electrophoresis. Although HPLC has been adapted for large-scale purification, it is highly expensive, requires expensive instrument and large volumes of harmful organic solvents, and is not suitable for high throughput purification. Gel electrophoresis is tedious, cannot be scaled up and is not suitable for high throughput purification either. Other methods for ODN purification include cartridge purification,[20,24-25] fluorous affinity purification[26-27] and biotin-streptavidin affinity purification.[28-30] To our knowledge, these methods have limited success on large-scale and high throughput purification although cartridge purification has been widely used for small scale purification. We recently reported a new technique for synthetic ODN purification, which we call catching full-length sequence by polymerization.[31-32] The method involves capping the failure sequences in each synthetic cycle with acetic anhydride or other reagents as in typical ODN synthesis, and tagging the desired full-length sequence with a polymerizable methacrylamide group at the end of synthesis. After cleavage and deprotection, the full-length sequence is co-polymerized into an insoluble cross-linked polyacrylamide polymer, while the failure sequences and other impurities remain in solution. After washing away the impurities with water, pure full-length ODN is cleaved from the polymer. In this paper, we report experimental procedures that prove the suitability of the technique for high throughput and large-scale ODN purification, and the unlimited power of the technology for isolation of long ODNs (up to 303-mer) from the complex mixtures of crude ODNs. In addition, to further confirm the high purity of the ODNs purified with the technique, several samples were thoroughly analyzed with LC-MS. This method represents a new dimension of analysis of the ODNs purified with the catching by polymerization techniques in the context of our previous analysis efforts.

Table 1. ODN numbering and length [a]

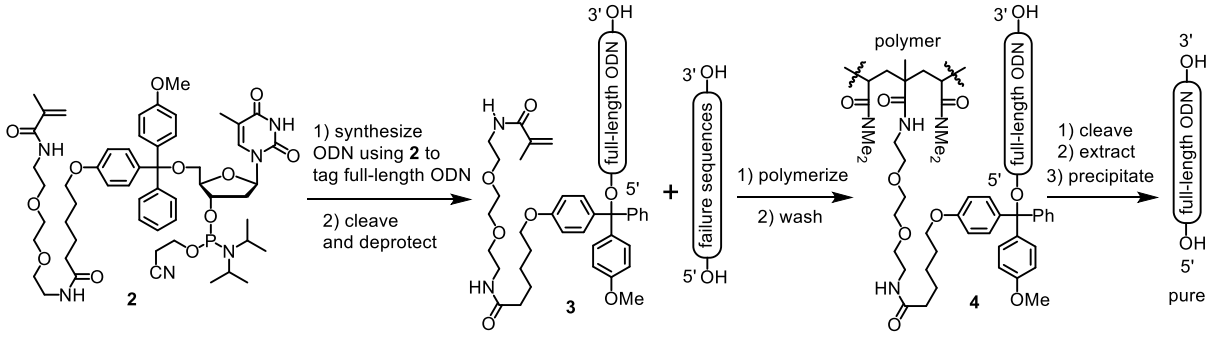
ODN	length	ODN	length
1a	15-mer	**1n**	43-mer
1b	21-mer	**1o**	64-mer
1c	23-mer	**1p**	80-mer
1d	19-mer	**1q**	90-mer
1e	20-mer	**1r**	110-mer
1f	20-mer	**1s**	151-mer
1g	20-mer	**1t**	197-mer
1h	21-mer	**1u**	203-mer
1i	21-mer	**1v**	225-mer
1j	22-mer	**1w**	251-mer
1k	26-mer	**1x**	275-mer
1l	28-mer	**1y**	303-mer
1m	32-mer		

[a] Sequence information is provided in Supporting Information.

RESULTS AND DISCUSSION

Parallel purification: To provide evidence that the technique is suitable for high throughput ODN purification, an experiment was set up to purify 12 different ODNs simultaneously. The ODNs (**1a**-**l**, Table 1) were selected from the Φ29 DNA polymerase gene. The equipment needed for the parallel purification were a multichannel pipette and a 96-well micro plate centrifuge. The ODNs were synthesized on an automated MerMade-6 synthesizer by standard DNA synthesis. Acetic anhydride was used to cap failure sequences during the synthesis. In the last synthetic cycle, the tagging reagent **2** instead of a standard nucleoside phosphoramidite was used for coupling (Scheme 1). Capping and oxidation were then carried out under normal conditions, but detritylation was omitted, which otherwise would remove the polymerizable methacrylamide tag introduce with **2**. After synthesis, the ODNs were cleaved from the solid support (CPG) and fully deprotected under typical conditions. The fully deprotected ODNs **3** had a polymerizable methacrylamide tag at their 5′-end (Scheme 1). The crude ODNs contained **3** and impurities including failure sequences and small molecules such as benzamide.

Scheme 1. The catching full-length sequence by polymerization ODN purification technique

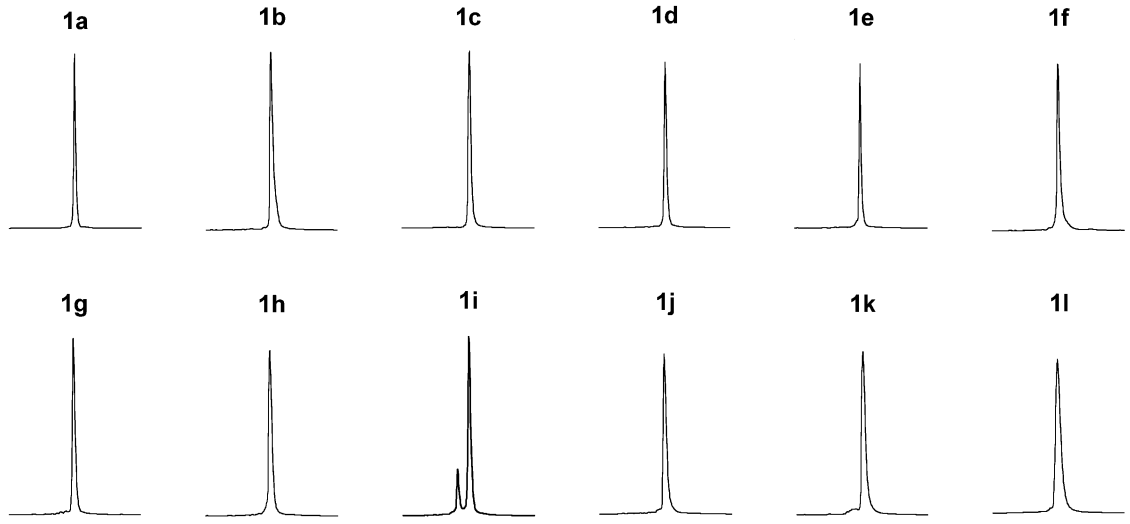


The parallel purification began after the solution of the 12 crude ODNs in concentrated ammonium hydroxide in 12 centrifuge tubes were evaporated to dryness in a vacuum centrifugal evaporator. Suitable volumes of water were added to the 12 tubes using the 12-channel pipette. The tubes were shaken gently to dissolve the ODNs. The polymerization solution containing *N*,*N*-dimethylacrylamide and *N*,*N*,*N*′,*N*′-methylenebis(acrylamide), and the polymerization initiation solutions of *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TMEDA) and ammonium persulfate were then added sequentially. The tubes were gently shaken, and the solutions were quickly transferred to the top compartment of 12 centrifugal filter units. The mixtures were allowed to polymerize over the filter in the units at room temperature for about one hour. The gels represented by **4** were broken into several pieces (Scheme 1). Water was added, and then removed by a short spin in a centrifuge. Several rounds of washing removed impurities including the failure sequences. To the gel was added 80% acetic acid, which cleaved the trityl ether bond in **4** and cleaved the full-length ODN from the polyacrylamide polymer. A short spin in a centrifuge separated the solutions from the gels. The gels were washed with water using the procedure involving adding water and spin for several times. The top compartment of the centrifugal filter units along with the gels in them were discarded and the solutions in the tubes were evaporated to dryness. The residue was dissolved in a small amount of ammonium hydroxide solution, and then *n*-butanol was added. The mixtures were then agitated and centrifuged. The full-length ODNs were precipitated. The supernatants were removed with the 12-channel pipettes. The precipitates were pure ODNs (Scheme 1). The steps involving precipitation with *n*-butanol from ammonium hydroxide solution may be omitted, and the purity of ODNs is not compromised according to HPLC analysis. However, we still suggest to carry out the precipitation procedure because it could remove any residue acetic acid from the ODNs, which could damage ODN over time.

The 12 purified ODNs were analyzed with RP HPLC. The critical sections of the HPLC profiles are shown in Figure 1. The full profiles of crude and pure ODNs are provided in Supporting Information. All ODNs were highly pure (> 99%) except for one of them. In the HPLC profile of **1i**, an additional small peak eluting before the major ODN peak was observed. The identity of this peak is unknown. We also noticed that in the profile of **1k**, there were some impurities before the ODN peak, which could be failure sequences. We believe that these small amount of impurities could be easily removed by washing the polyacrylamide gel **4** more thoroughly. For all the 12 ODNs, MALDI-TOF MS analyses gave predicted molecular peaks (Supporting Information). It is remarkable that in all the cases, the spectra were very clean.

Except for a few minor peaks, all appreciable peaks were accountable based on the ODN structures (Supporting Information).

Figure 1. Critical sections of the HPLC profiles of ODNs **1a-l**. The ODNs were purified in a high throughput fashion using the catching full-length sequence by polymerization technique.

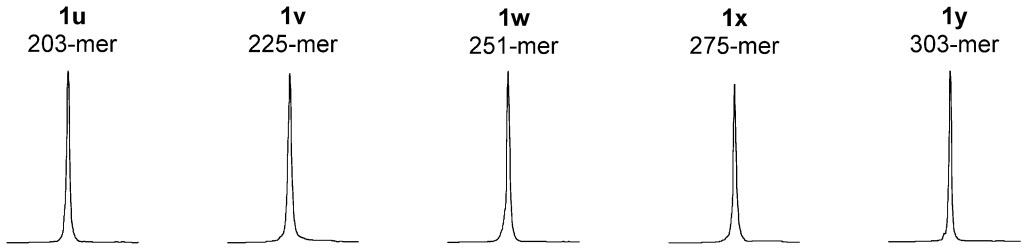


The protocol described above for the parallel purification of 12 ODNs does not contain any complicated manipulations, and all the steps could be performed in a parallel fashion. Therefore, in principle, the purification technique can potentially be scaled up to purify 96 or more ODNs simultaneously without significantly increasing the working load. Because high throughput ODN synthesis technologies have been developed in the past few years, we believe that our catching by polymerization technique could bring about transformative changes in the industry of high throughput ODN production.

Large-scale purification: To prove that the catching by polymerization technique is suitable for large-scale ODN purification, we synthesized the 32-mer ODN **1m** at 60 μmol scale. The synthesis conditions were similar to those for the synthesis of the ODNs for parallel purification but with some modifications including carrying out the synthesis using two 30 μmol columns. Experimental details are disclosed in the experimental section. The catching by polymerization procedure was also similar to the parallel purification except that the polymerization was performed in a round bottom flask and the polyacrylamide gel (**4**) was transferred into a Büchner funnel with glass sintered filtration disc for washing away impurities and cleaving ODN from the gel. The former was achieved with trimethylamine solution and water. The latter was conducted with 80% acetic acid. In the processes, removing liquids from the funnel was achieved by applying vacuum from a water aspirator. The filtrate containing the full-length ODN was evaporated to dryness, which gave a light yellow sticky oil. Upon precipitation from concentrated ammonium hydroxide by *n*-butanol, the ODN appeared as a white solid (photo in Supporting Information). HPLC analysis indicated that the ODN was highly pure (Supporting Information). The OD_{260} was determined to be 13,075, which corresponds to a 65% yield for the synthesis and purification. The yield was significantly higher than what we obtained earlier (15%).[31] The

improved yield was likely a result of more efficient coupling of **2** to the 5'-end of ODN during synthesis and more complete extraction of the full-length ODN from the gel during purification.

Figure 2. Critical sections of the HPLC profiles of the long ODNs **1u**-**y**. The ODNs were purified using the catching full-length sequence by polymerization technique.



Long ODN synthesis and purification: A highly impressive feature of the catching full-length sequence by polymerization technique is its power to purify long ODNs. We believe that as long as an ODN can be synthesized, no matter how long it is and how complex the crude mixture is, using the technique, the full-length ODN could potentially be isolated. We previously demonstrated the isolation of ODNs as long as 197-mer from complex crude mixture.[31] In the current studies, we successfully synthesized and purified ODNs **1n**-**1y**, which ranged from 43-mer to 303-mer (Table 1). The ODN synthesis conditions were similar as described for the synthesis of ODNs for parallel purification with some modifications. The syntheses were conducted on CPGs with larger pore sizes (1400 Å and 2000 Å) and at smaller scales (0.2 μmol). Before synthesis, the CPG was subjected to the capping conditions to block any free hydroxyl and amino groups. During synthesis, coupling was conducted three times instead of two. It is noted that during the long ODN syntheses, when the ODNs reached the length of 140-nucleotide, no red or orange color of the trityl cation could be observed in the synthesis column during detritylation. The synthesizer could not detect any trityl signal either. However, we assumed that a small portion of the nucleotide chains were still not capped and could continue to grow in subsequent synthetic cycles. We therefore allowed the synthesis to continue.

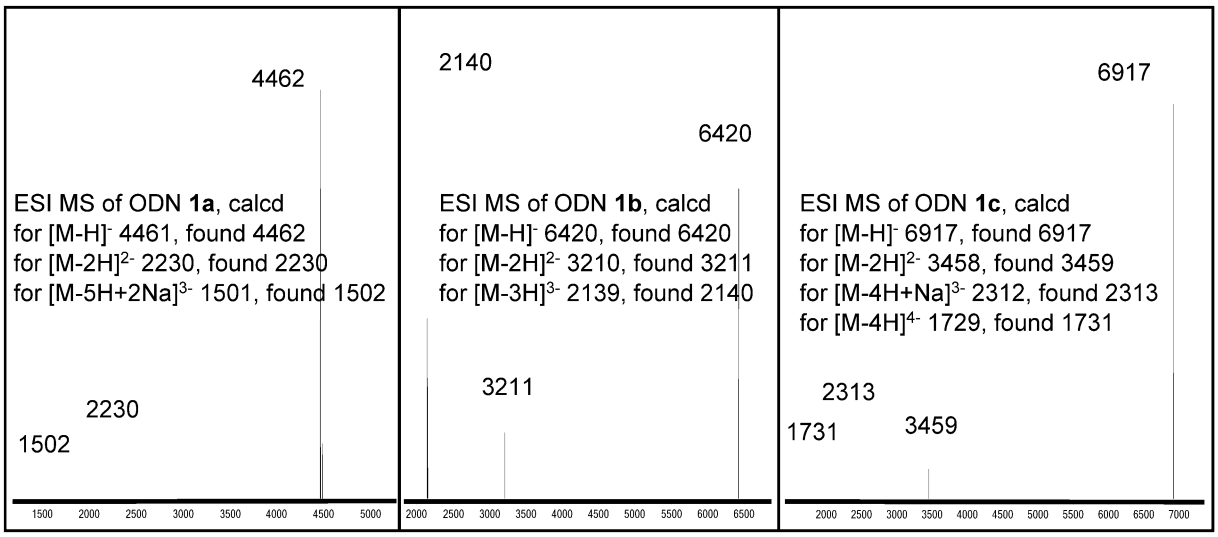
We were not certain if we could isolate ODNs longer than 200-mers and detect them with HPLC. Therefore, we gradually increased the length from 203-mer (**1u**), 225-mer (**1v**), 251-mer (**1w**), 275-mer (**1x**), to 303-mer (**1y**). After successful synthesis and purification of shorter ones in the series, we went further to the next longer ones. The purification procedure was similar as described for parallel purification except that the ODNs were purified one at a time. In addition, urea was added to the polymerization solution to destroy any possible secondary structures of the long ODNs or hybrids formed between the long ODNs and failure sequences. Destroying these structures is important for washing away impurities efficiently. Overall, in all the purification cases, single sharp peaks of ODNs were observed in the HPLC profiles. The critical sections of the HPLC profiles of **1u**-**y** are shown in Figure 2. The full profiles of crude and pure **1n**-**y** are provided in Supporting Information.

We believe that the success of synthesizing ODNs with lengths up to 303-nucleotide and using the catching full-length sequence by polymerization technique to isolate them from the complex crude mixture is noteworthy. To our best knowledge, so far no other technique has the

capability to isolate target ODNs from the complex mixtures generated from the many steps of chemical synthesis needed for assembling the long ODNs. In principle, gel electrophoresis could purify 303-mer ODNs because it could resolve a 303-mer from a 302-mer. However, when the mixture is highly complex and the mass percentage of the desired full-length ODN in the mixture is low, gel electrophoresis is impractical for the application. The solid phase extraction approach, which relies on the formation of a covalent bond between a functionalized solid support and the full-length ODN, could be an option. However, reactions between large molecules are inherently slower than the same reactions between smaller molecules, and it is difficult for large ODNs to diffuse into the matrix of solid phase to form the covalent bond, this approach is unlikely to achieved satisfactory results for long ODN purification either.[33] The purification approaches based on the interactions between antigen and antibody such as biotin and streptavidin could also suffer the problem of difficulty for large molecules to diffuse into solid matrix.[28-30] The fluorous affinity purification method has been demonstrated for long ODN purification, but when the length of the ODNs reaches 100-mer, differentiation between the full-length sequence and failure ones becomes difficult.[26-27] Other methods such as HPLC and hydrophobic cartridge purification are clearly unsuitable for the application. When considering technologies for long ODN purification, it is important to note that resolution of ODNs of different lengths is only one of the challenges. Another challenge, which is to isolate the desired sequence that is of low mass percentage from a complex mixture, is even more difficult to overcome. The catching full-length sequence by polymerization technique is well suited to overcome both of the challenges, and therefore it is a promising tool for accomplishing this difficult task.

LC-MS analysis of the ODNs purified with the catching-by-polymerization technique: One concern about the catching by polymerization purification technique is the damage of ODNs in the acrylamide radical polymerization step. So far, we have used RP HPLC, anion-exchange HPLC and MALDI-TOF MS to characterize the ODNs. No ODNs that were damaged by radicals were detected using these analytical methods.[31-32,34-36] In addition, we subjected the four nucleosides – adenosine, cytidine, guanosine and thymidine – to the acrylamide radical polymerization conditions; HPLC analyses of the recovered nucleosides showed that they were identical with authentic nucleosides, and the recovered guanosine was different from 8-oxo-guanosine – the commonly observed radical damaged guanosine.[32] We had synthesized ODNs containing 8-oxo-guanosine, and compared them with the ODNs synthesized with unmodified nucleosides and purified with the catching by polymerization technique, no damage in the purified ODNs was found either.[37] We also conducted the experiments involving enzymatic digestion of the purified ODNs into nucleotides, converting the nucleotides to nucleosides, and HPLC analysis of the nucleosides; HPLC analysis indicated that the nucleosides were intact.[37] However, ODNs are relatively large molecules, their characterization, especially determining their purity, is highly challenging. Therefore, more dimensions of characterization of the ODNs purified by the catching by polymerization technique is always desirable. For this reason, we selected three sequences (**1a**-**c**) from the ODNs purified using the catching by polymerization technique, and analyzed them with LC-MS.

Figure 3. Critical sections of the MS spectra of ODNs **1a**-**c**. The sections are from full MS spectra obtained from the full elution peak range in LC-MS experiments. More detailed LC-MS data are provided in Supporting Information.



The critical sections of the MS spectra of the three ODNs (**1a**-**c**) obtained from the full elution peak range are shown in Figure 3. The full MS spectra from the full elution peak range as well as selected sliced LC peak ranges are provided in Supporting Information. Overall, all the spectra were very clean. Except for peaks from double or triple charged full-length ODNs, additional peaks with masses lower than the predicted molecular masses were not observed or very insignificant. Additional peaks with masses higher than the predicted molecular weight other than common metal adducts of the ODNs were not detected in all the three cases. These observations indicated that all failure sequences and other impurities in the crude ODNs were washed away during purification. In addition, the n-1 deletion sequences and n+1 addition sequences, which could be resulted from incomplete capping of failure sequences and double coupling due to premature detritylation, respectively, were not formed in any appreciable amounts during ODN synthesis. Importantly, the results once again prove that the conditions for the acrylamide radical polymerization reaction during purification did not cause ODN damages.

CONCLUSION

To advance the catching full-length sequence by polymerization technique to the level of practical use for ODN purification, we successfully adapted the technique for parallel purification by purifying 12 ODNs simultaneously. In addition, we further demonstrated the suitability of the technique for large-scale purification and the power of the technique for long ODN purification. It is remarkable that the technique can readily isolate ODNs up to 303 nucleotides in length with low mass percentages from highly complex crude mixtures. Further, we used LC-MS to analyze three ODNs purified with the catching by polymerization technique. This represents an additional dimension of characterization of the ODNs purified with the technique. The results further proved that the ODNs were highly pure. Currently we are making efforts to identify a

niche market for the catching by polymerization technique to enter the field of ODN production. We believe that the technique will be equally suitable for purification of RNA and other biooligomers including peptides,[38] and we are also making efforts in those areas.

EXPERIMENTAL SECTION

General Information. Reagents and CPG for ODN synthesis were purchased from Glen Research and Bioautomation, and were used as received. The polymerizable tagging agent **2** was synthesized according to reported procedure.[31] Chemicals used in the catching by polymerization procedure were purchased from Aldrich, and were used directly. The centrifugal filtering unit was purchased from Aldrich. Analytical RP HPLC was performed on a JASCO LC-2000Plus System with PU-2089Plus Quaternary Gradient pump and UV-2075Plus detector. Column: C-18, 5 μm, 100 Å, 250 × 3.20 mm. Detection: UV at 260 nm. Eluents and gradient for pure and crude **1a**-**m** and crude **1n**-**y**: solvent A, 0.1 M triethylammonium acetate and 5% acetonitrile; solvent B, 90% acetonitrile; time, 0-60-80 min, B%, 0-45-100; flow rate 1.0 mL/min. Eluents and gradient for pure **1n**-**y**: solvent A, 200 mM HFIP and 8.1 mM Et_3N; solvent B, methanol; time, 0-5-25-80 min, B%, 5-5-70-70; flow rate 1 mL/min. MALDI-TOF MS were obtained on Bruker's microflex™ LRF MALDI-TOF System.

ODN Synthesis: All ODNs were synthesized on a MerMade 6 solid phase synthesizer. The synthesizer manufacturer recommended synthesis scripts were used with slight modifications. Concisely, long chain amino alkyl CPG (pore size, 1000 Å for ODNs **1a**-**m**, 1400 Å for **1n**-**r**, 2000 Å for **1s**-**y**) was used as solid support. Detritylation: 2% DCA in DCM, 90 sec × 2 (50 sec × 4 for **1m**). Coupling: 0.1 M commercial 2-cyanoethyl 5′-DMTr Bz-dA,Ac-dC, *i*Bu-dG and dT in acetonitrile, 100 sec × 2 (100 sec × 3 for **1m**-**y**). Capping: cap A, THF/pyridine/Ac_2O (8:1:1), cap B, 16% methylimidazole in THF, 50 sec × 2 (50 sec × 4 for **1m**). Oxidation: 0.02 M I_2 in THF/pyridine/H_2O, 60 sec (90 and then 25 sec, and repeated 1 time for **1m**). In the last synthetic cycle, the tagging phosphoramidite **2** instead of a standard nucleoside phosphoramidite was used in the coupling step (100 sec × 5 for **1m**, 100 sec × 3 for others). At the end of the synthesis, detritylation was not performed. Special for **1n**-**y**, before synthesis, the CPG was subjected to capping (50 sec × 2) to block any free hydroxyl and amino groups.

Parallel Purification. The ODNs **1a**-**l** were synthesized on a 1 μmol scale under conditions described in ODN Synthesis. The CPGs were divided into 5 equal portions. One portion was suspended in conc. NH_4OH (300 μL) in sealed 1.5 mL centrifuge tubes and heated at 55 °C for 12 h. After cooling to rt and removing supernatants, the CPGs were washed with conc. NH_4OH (100 μL). The combined supernatants and washes for each ODN were evaporated to dryness in a vacuum centrifugal evaporator. To the ODNs in 12 centrifuge tubes were added 50 μL water using a 12-channel pipette. The tubes on a 96-well plate were shaking gently to dissolve the ODNs. A small portion of the solution (1 μL) was injected into HPLC to generate the crude profiles. A polymerization solution (12 μL) containing *N*,*N*-dimethylacrylamide (6.6 M) and *N*,*N*′-methylenebis(acrylamide) (0.33 M) was added to the remaining solution. After shaking the plate gently, the polymerization initiator solutions *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (0.66 M, 5 μL) and $(NH_4)_2S_2O_4$ (0.22 M, 5 μL) were added sequentially. The 12 solutions were transferred to the top compartment of 12 centrifugal filter units using the 12-channel pipette. The tubes were closed and the mixtures were allowed to polymerize at rt for ~1 h. The polyacrylamide gels were

loosened or broken into pieces, and NaOAc solution (20%, 250 μL or more) was added, which was removed with a spin in a centrifuge after standing for 3 min. The washing was repeated 8 times. Then the gels were washed with a Et_3N solution (5%, 250 μL or more, 3 min) for 6 times, and finally with water (250 μL or more, 0 min standing time) for 6 times. All the filtrates were discarded. To the gel was added minimum volumes of 80% AcOH that could cover the gel (~100 μL). The cleaving reaction was allowed to proceed at rt for 3-5 min. The cleaving solutions were collected by spinning in a centrifuge. The cleavage procedure was repeated 3 times. The gels were washed with water (~100 μL × 2, 3-5 min standing for each), and the combined cleaving solutions and washes were evaporated to dryness in a vacuum centrifugal evaporator. To the ODNs were added conc. NH_4OH (100 μL). After warming at 65 °C for 10 min and cooling to rt, *n*BuOH (900 μL) was added. The tubes were vortexed (~20 sec), and then centrifuged at ~14K rpm for ~3 min. The supernatants were removed with the 12-channel pipette, and the residues were the purified ODNs. For HPLC analysis, to the ODNs were added 50 μL water, and 20 μL was inject into HPLC to obtain the profiles of pure ODNs. The OD_{260} values of the ODNs were obtained as described in Supporting Information.

Large-Scale Purification. The ODN **1m** was synthesized using two 30 μmol synthesis columns under conditions described in ODN Synthesis. Cleavage and deprotection were carried out in a sealed pressure tube with conc. NH_4OH (25 mL, 65 °C, 12 h). The CPG and ODN solution were separated by filtration, and the CPG was washed with NH_4OH (5 mL × 5). The solution and washes were combined in a round bottom flask. A small portion (1 μL) was used to generate the crude HPLC profile. After adding ~1 mL DIEA to the remaining solution, the mixture was concentrated to close to dryness on a rotary evaporator under high vacuum. To the ODN was added water (3.4 mL), *N*,*N*-dimethylacrylamide (2.27 mL), and *N*,*N*′-methylenebis(acrylamide) (70 mg), and the flask was sealed with a rubber septa and placed under nitrogen from a gas line via a needle. The flask was shaken gently for the contents to form a homogenous solution. The nitrogen line was shifted to vacuum from a water aspirator to degas the solution briefly, and the line was shifted back to nitrogen. The initiator solutions *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (0.66 M, 10 μL) and $(NH_4)_2S_2O_4$ (0.22 M, 10 μL) were added via syringes. The flask was shaken shortly to mix the contents, and then allowed to stand at rt under nitrogen. After 1 h, a gel was formed, which was broken into small pieces and transferred into a 60 mL Büchner funnel with a glass sintered filtration disc. The gel was washed with 5% Et_3N (15 mL × 12, 30 min incubating for each wash), and then water (20 mL × 12, 0 min incubating). The ODN was then cleaved from the gel with 80% AcOH (10 mL × 5, rt, 10 min incubating each time). The gel was washed with water (10 mL× 8, rt, 15 min incubating). The gel was further soaked in water overnight (80 mL, rt), from which significant amount of ODN was recovered. Additional extraction of the gel with water did not give any ODN. All the solutions containing ODN including the AcOH solution, the water washes and soaking solution were combined and evaporated to dryness. The residue was dissolved in conc. NH_4OH (5 mL), *n*BuOH (45 mL) was added. Upon mixing, a white solid was formed. The supernatant was removed. The solid was dissolved in water and transferred into a centrifuge tube. Volatiles were removed under vacuum in a centrifugal evaporator giving the ODN as a white solid. The ODN was dissolved into water (5 mL). A small portion was injected into HPLC to generate the pure profile. The OD_{260} value of **1m** was obtained as detailed in Supporting Information.

Long ODN Purification. The long ODNs **1n**-**y** were synthesized at 0.2 μmol under the conditions described in ODN Synthesis. Cleavage and deprotection were achieved using conc.

NH_4OH at 55 °C for 12 h. The catching by polymerization purification procedure was similar as described for parallel purification with the following modifications. The ODNs were purified one by one instead of simultaneously. Before adding the polymerization solution, the ODNs were dissolved in a urea solution (7 M, 50 μL) instead of water. After precipitation with *n*BuOH, the ODNs were dissolved in water (100 μL), heated to 60 °C, and cooled to 0 °C rapidly. Immediately, 20 μL of the solution was injected into HPLC to obtain the pure HPLC profiles. The OD_{260} values of the ODNs were obtained as detailed in Supporting Information.

LC-MS Analysis. All analyses were performed on an Agilent 1200 HPLC System, which was coupled to an Agilent 6224 Time of Flight LC/MS system with Electrospray Ionization source. Column: Agilent Extended C-18, 1.8 μm, 80 Å, 50 × 2.1 mm. Detection: negative total ion content. Eluents and gradient: solvent A, 200 mM HFIP and 8.1 mM TEA; solvent B, methanol; time, 0-1-11-11.5-14.5-15 min; B%, 10-10-70-90-90-10; flow rate 0.2 mL/min. Column temperature: 40 °C. Critical sections of MS of full elution peak ranges of ODNs **1a**-**c** are in Figure 3. Full profiles of LC, and full MS spectra of full elution peak and selected sliced peak ranges are in Supporting Information.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI:

ODN sequences, HPLC profiles, MALDI MS, photo of **1m**, LC-MS, and UV spectra and OD_{260}.

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Notes
The authors intend to commercialize the purification technology.

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