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Oligonucleotide synthesis under mild deprotection conditions

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Abstract

Over a hundred non-canonical nucleotides have been found in DNA and RNA. Many of them are sensitive toward nucleophiles. Because known oligonucleotide synthesis technologies require nucleophilic conditions for deprotection, currently there is no suitable technology for their synthesis. The recently disclosed method regarding the use of 1,3-dithian-2-yl-methyl (Dim) for phosphate protection and 1,3-dithian-2-yl-methoxycarbonyl (Dmoc) for amino protection can solve the problem. With Dim-Dmoc protection, oligodeoxynucleotide (ODN) deprotection can be achieved with $NaIO_4$ followed by aniline. Some sensitive groups have been determined to be stable under these conditions. Besides serving as a base, aniline also serves as a nucleophilic scavenger, which prevents deprotection side products from reacting with ODN. For this reason, excess aniline is needed. Here, we report the use of alkyl Dim (aDim) and alkyl Dmoc (aDmoc) for ODN synthesis. With aDim-aDmoc protection, deprotection is achieved with $NaIO_4$ followed by K_2CO_3. No nucleophilic scavenger such as aniline is needed. Over 10 ODNs including one containing the highly sensitive N^4-acetylcytidine were synthesized. Work on extending the method for sensitive RNA synthesis is in progress.

Introduction

Many non-canonical nucleotides have been found in DNA and RNA. They serve as an additional layer of regulation of the flow of genetic information in biological systems. Malfunction of this layer of regulation has been found relating to many human diseases.[1] In addition, modified nucleotides can also be produced by reactions of DNA with DNA modification drugs and toxic compounds from the environment. These DNA adducts have been suggested to be the cause of many cancers.[2] To study the functional mechanisms of the modified nucleotides including those that cause human diseases, chemical synthesis of oligodeoxyribonucleotide (ODN) and oligoribonucleotide (ORN) containing such modified nucleotides is needed. However, known oligonucleotide (ON) synthesis methods use protecting groups and linkers that require harsh basic and nucleophilic conditions for cleavage and deprotection. Many of the modified nucleotides are sensitive to such conditions, and thus ONs containing them cannot be synthesized using known synthesis methods.

Some existing methods could be considered for the synthesis of ONs containing sensitive nucleotides. However, all of them have limitations. For example, using the phenoxyacetyl based groups for amino protection, ON deprotection can be achieved under milder conditions than using typical acyl groups. However, the conditions such as dilute sodium methoxide in methanol and concentrated

ammonium hydroxide at room temperature are still strongly basic and nucleophilic.[3] Using cleavable linkers based on nitro benzyl groups, ON cleavage can be achieved under non-nucleophilic and non-basic photolytic conditions. However, it is documented that UV light can damage ON.[4] Using allyl and benzyl based groups for amino protection, ON deprotection can be achieved using palladium. However, excess palladium has to be used, and palladium is toxic, difficult to remove, and expensive.[5] The (*p*-nitrophenyl)ethyl (Npe) and (*p*-nitrophenyl)ethyloxycarbonyl (Npeoc) groups were studied for ON synthesis, but deprotection of these groups requires the strong base DBU with a nucleophilic scavenger.[6] Several other methods in the literature can also be considered for sensitive ON synthesis, which are summarized elsewhere.[7] Again, all of them have limitations.

Most recently, Meier's research group at NCI used the cyanoethyloxycarbonyl (Ceoc) group and a photo-cleavable linker for the synthesis of ORNs containing the sensitive ac^{4}C function.[8] Deprotection and cleavage were achieved under non-nucleophilic conditions in three steps consisting base treatment, fluoride treatment and photo irradiation. The work constitutes a significant progress in the field. However, many challenges remain. The longest ORN reported was a 15-mer along with several 10-mer ORNs. The guanosine nucleotide was incorporated with a phosphoramidite without amino protection, which requires careful control of coupling conditions to avoid *N*-phosphitylation. The use of an unprotected phosphoramidite also prevents capping failure sequences during solid phase synthesis, which could put a limit on the length of ORN that can be synthesized, and may require the ORN product to be purified with gel electrophoresis instead of the more scalable HPLC. In addition, the deprotection needs to use DBU, which prevents the method for the synthesis of ORN containing some sensitive nucleosides such as mchm^{5}U. Therefore, developing new methods for sensitive ON synthesis remains a significant and challenging task.

In 2016, we published our first paper on sensitive ODN synthesis.[9] We used the Dmoc group for amino protection and a Dmoc linker to anchor ODN to solid support. ODNs synthesized can be represented by **1a** (Scheme 1). Deprotection and cleavage were achieved in three steps by removing the 2-cyanoethyl phosphate protecting group with DBU, oxidation of the Dmoc functions with sodium periodate and aniline induced β-elimination. Besides serving as the base for β-elimination, excess aniline was used as a nucleophilic scavenger for the deprotection side product **2hh**, which would otherwise react with the deprotected ODN via Michael addition (Scheme 2). ODNs containing sensitive groups such as alkyl ester, aryl ester, thioester, alkyl halide, α-halo amide and chloropurine were successfully synthesized using the method.

To avoid the use of excess aniline, dmDmoc group was tested for amino protection.[10] The ODN synthesized can be represented with **1b**. Deprotection and cleavage were achieved under similar conditions except that the excess aniline could be replaced with dilute potassium carbonate at pH 8. The deprotection side product **2mm** was found unreactive toward deprotected ODNs due to steric hindrance. Unfortunately, the dmDmoc protection was not completely stable under the acidic detritylation conditions, and as a result, significant amounts of branched ODNs were formed although the desired ODN could be purified using RP HPLC.[10] Later, we tested the used of Dim for phosphate protection and Dmoc for amino protection.[11] The ODNs synthesized can be represented by **1c** (Scheme 1). Deprotection and cleavage were achieved in two steps by oxidation with sodium periodate followed by excess aniline.

In this paper, we report the study of alkyl Dim (aDim) and alkyl Dmoc (aDmoc) as protecting groups for sensitive ODN synthesis. The ODNs synthesized can be represented by **1d**. We found that deprotection can be achieved in two steps by oxidation with sodium periodate followed by β-elimination with potassium carbonate (Scheme 1). The deprotection side products **2pe**, **2pr** and **2me**, although less

hindered than **2mm**, were found unreactive toward deprotected ODN, which indicates that the reaction in Scheme 2 favours the left side. As expected, the aDim and aDmoc groups, unlike the dmDmoc group that can form a tertiary carbocation under acidic conditions, were completely stable under the detritylation conditions. Over 10 ODNs including one containing the sensitive N^4-acetyldeoxycytidine group were synthesized and readily purified with RP HPLC.The ODNs were characterized with HPLC and MALDI MS and in selected cases capillary electrophoresis.

Results and Discussion

peDim-peDmoc phosphoramidites for ODN synthesis: We hypothesized that using the phosphoramidites **5dNpe** (N = nucleoside T, C, A or G; Figure 1) for ODN synthesis, the ODN deprotection side product **2pe**, due to its steric hindrance from the pentyl group, might not react with the deprotected ODN to form adduct **4** (Scheme 2) in the absence of any nucleophilic scavenger. We further hypothesized that even if the steric hindrance were not enough to suppress the side reaction, because ODN deprotection is performed in water, the hydrophobicity of the pentyl group could make **2pe** barely soluble in water, which could shift the Michael addition side reaction toward free ODN (Scheme 2). For these reasons and considering the low chance of instability of the peDim and peDmoc protections under the acidic detritylation conditions during ODN synthesis, we decided to investigate the effectiveness of using peDim and peDmoc as protecting groups for ODN synthesis.

The synthesis of the phosphoramidite monomers **5dNpe**, which includes **5dTpe**, **5dCpe**, **5dApe** and **5dGpe**, is shown in Scheme 3. The required reagents **8a** and **9a** were readily synthesized from **6** using similar procedures we reported previously.[11a] For **9a**, due to its sensitivity to oxygen and moisture, it was synthesized at the time of use and used for the next reactions without purification and characterization.

Compound **5dTpe** was synthesized under standard phosphitylation conditions we reported previously for the synthesis of Dim-Dmoc phosphoramidites from **10** in 86% yield (Scheme 3).[11a] We also synthesized **13dTpe**, which carries a 5'-Tr group instead of a 5'-DMTr group. The compound was needed for the incorporation of the last nucleoside in ODN synthesis to assist RP HPLC purification of the ODN product. Our earlier studies indicated that the DMTr group could not survive the slightly acidic sodium periodate oxidation conditions for ODN deprotection.[10] For the synthesis of **5dCpe** and **5dApe**, the introduction of peDmoc to the amino group and the tritylation of 5'-OH group to give **17a** and **21a** were carried out smoothly under conditions similar to our previously reported conditions used for the synthesis of dmDmoc phosphoramidites.[10] For the synthesis of **5dGpe**, the introduction of peDmoc and DMTr to give **26a** were also similar to that for the synthesis of dmDmoc-dG amidites.[10] However, we improved the synthesis by the use of the bulkier TBDPS group instead of the TBS group for the protection of the lactam in the nucleobase. When TBS was used, the product was too labile toward hydrolysis to be isolated. With TBDPS, the product **23** could be isolated in good yield, which made the subsequent reaction for the installation of the peDmoc group more reproduceable. With compounds **17a**, **21a** and **26a**, the synthesis of the corresponding **5dNpe** phosphoramidites were carried out smoothly using our previously reported procedure (Scheme 3).[11a]

ODN synthesis using the **5dNpe** phosphoramidites was carried out under standard conditions with a few modifications.[11a] Capping failure sequences was conducted using 2-cyanoethyl *N*,*N*,*N*′,*N*′-tetraisopropylphosphorodiamidite with 4,5-dicyanoimidazole as the activator. In the last synthetic cycle, phosphoramidite **13dTpe** was used so that the full-length sequence was tagged with a trityl group instead of a DMTr group.[10] The resulting ODN can be represented by **27** (Scheme 4). Deprotection and cleavage were achieved in two steps. In the fist step, the sulfide groups in the peDim and peDmoc

protecting groups and the Dmoc linker were oxidized with a sodium periodate solution (0.4 M), which has a pH of 4 in the absence of any added acid, at room temperature to give **28**. In the second step, β-elimination was induced with potassium carbonate (0.05%), which has a pH of 8, at room temperature to give fully deprotected ODN (**3**). The total deprotection and cleavage time was about 6 hours, which could be shortened by raising reaction temperature. However, we do not suggest doing so because ODNs containing sensitive groups could decompose. The ODN was then purified with Tr-on RP HPLC, and the pure ODN was analyzed with MALDI MS.

Using the procedure, three 11-mer ODNs (**29a-c**, Figure 2) were synthesized. Their crude and pure HPLC profiles and images of MALDI MS are given in ESI. The RP HPLC profile of crude **29c** is also shown in Figure 3a, which shows that the synthesis was efficient as only one major peak corresponding to Tr-tagged full length sequence appeared (retention time ~40 minutes). The HPLC profile of crude **29c** from detritylation of RP HPLC purified tritylated ODN is shown in Figure 3b. The MALDI MS of the purified detritylated **29c** is shown in Figure 3c. The mass of the molecular ion matches the calculated value.

Encouraged by the results, we proceeded to use the method to synthesize longer ODNs such as 20-mers. However, RP HPLC analysis found that complex mixtures were formed. Careful manipulations such as paying extra attention on drying phosphoramidite monomers and using longer coupling times did not improve the synthesis. After many trials, we concluded that the hydrophobic pentyl groups in the **5dNpe** phosphoramidites may have an adverse effect on ODN synthesis. When the ODN on the solid support reached certain length, the pentyl groups might reduce the solubility of the protected ODN on the solid support, and as a result, the reactions such as coupling and oxidation were inefficient.

prDim-prDmoc phosphoramidites for ODN synthesis: With the reasoning that the hydrophobicity of the pentyl group in **5dNpe** may have an adverse effect on ODN synthesis, we next synthesized the **5dNpr** phosphoramidites (Figure 1 and Scheme 3). We hypothesized that the propyl group, which is less hydrophobic than the pentyl group, in the aDim and aDmoc groups could have a less adverse effect on ODN synthesis, while it is still sufficiently hydrophobic as well as hindered to prevent the Michael addition side reaction between **2** and **3** (Scheme 2) during ODN deprotection. The phosphoramidites were synthesized using the same procedure for the synthesis of **5dNpe** (Scheme 3). Good to excellent yields were obtained. Indeed, using these monomers, under the conditions described above for ODN synthesis using **5dNpe**, we were able to synthesize longer ODNs (**29d-f**), which included two 19-mers. It is noted that potassium carbonate was used to induce β-elimination during deprotection and cleavage. There was no need of nucleophilic scavenger such as aniline to suppress the Michael addition side reaction (Scheme 2). The crude and pure HPLC profiles and MALDI MS image of **29e** are shown in Figure 3d-f. Additional analytical data including those for **29d-f** are provided in ESI. As can be seen, the syntheses had good yields and the full-length sequences were easy to purify. The results indicate that the hydrophobicity of the pentyl group in **5dNpe** may indeed have an adverse effect on ODN synthesis, and the propyl group in **5dNpr** have less such adverse effect.

meDim-meDmoc phosphoramidites for ODN synthesis: Encouraged with results using phosphoramidites **5dNpr**, we were interested in knowing if phosphoramidites **5dNme** would be suitable for ODN synthesis. In this case, the hydrophobicity of methyl group would have much less effect on the equilibrium of the Michael addition side reaction involving **2**, **3** and **4** (Scheme 2), and there is a possibility that the ODN adduct **4** could be formed in significant quantity rendering the need of a scavenger such as aniline. If that were the case, aDim-aDmoc would not offer any advantage over Dim-Dmoc we reported earlier.[11a] However, if the steric hindrance from the methyl group alone or together with the limited hydrophobicity provided by the methyl group can be sufficient to suppress the

formation of **4**, phosphoramidites **5dNme** would have significant advantages over other aDim-aDmoc phosphoramidites. They are more atom economic, and their lower hydrophobicity may have no or little effects on the efficiency of ODN synthesis. With these considerations, we decided to synthesize **5dNme** phosphoramidites.

As shown in Scheme 3, the procedure for the synthesis of **5dNme** were the same as that for the synthesis of **5dNpe** and **5dNpr**. The yields for the transformations were good to excellent. Using these less hydrophobic phosphoramidites, we successfully synthesized ODNs **29g-h**, among which **29h** contains 23 nucleotides. The conditions for ODN synthesis, and deprotection and cleavage as well as HPLC purification were the same as described for the synthesis of ODNs **29a-f** (Scheme 4). No scavengers such as aniline was needed to suppress the Michael addition side reaction (Scheme 2). The HPLC profiles of crude and pure ODN **29h** are shown in Figure 3g-h. The image of its MALDI MS is also given (Figure 3i). More HPLC and MALDI MS data for the ODNs are provided in ESI. As can be seen, the ODNs synthesized is easy to purify as the trityl tagged full-length sequence is well separated from impurities in the RP HPLC profile. The results confirm that the steric hindrance of the methyl group along with the limited hydrophobicity from the methyl group in **5dNme** is sufficient to suppress the Michael addition side reaction between **2** and **3** (Scheme 2). Therefore, due to the advantages of methyl group over propyl and pentyl groups discussed earlier, we conclude that **5dNme** phosphoramidites are the best choice for sensitive ODN synthesis using the aDim and aDmoc protecting groups.

CE-meDmoc phosphoramidites for ODN synthesis: Although phosphoramidites **5dNme** would be more ideal for sensitive ODN synthesis than the CE-meDmoc phosphoramidites **30a-c** (Scheme 5), their synthesis requires the preparation of the oxygen and moisture sensitive phosphitylation agent **9c**, which may be challenging for inexperienced individuals. Because CE-meDmoc phosphoramidites are expected to meet most of the needs of sensitive ODN synthesis and their synthesis is much easier due to the commercial availability of the phosphitylation agent **11**, we decided to synthesize **30a-c**, and demonstrate their use for sensitive ODN synthesis.

With the availability of **17c**, **21c**, and **26c**, phosphoramidites **30a-c** were synthesized under standard conditions using the commercially available phosphitylation agent **31** (Scheme 5). The ODNs **29i** was synthesized using **30a-d** under the same conditions described for **29a-h** except that the last nucleotide at the 5'-end was incorporated with **30e**. The coupling yields ranged from 86% to 100%. A trityl assay log is included in the ESI, which is typical for the successful ODN syntheses in this article. The ODNs synthesized can be represented with **32** (Scheme 6). Deprotection and cleavage were achieved in three steps. First, the 2-cyanoethyl group was removed with DBU at room temperature; second, the sulfides in meDmoc and linker were oxidized with sodium periodate; and third, the oxidized meDmoc and linker were cleaved with potassium carbonate. The HPLC profiles, capillary electrophoresis profile, and MALDI MS of ODN **29i** are provided in ESI. As can be seen, the ODNs are easy to purify as the Tr-tagged full length sequences are well separated from impurities in the RP HPLC profiles, and ODNs with good purity can be obtained.

We were interested in testing if the meDmoc groups and the Dmoc linker in ODNs **32** could be cleaved by a non-nucleophilic base such as DBU without oxidizing the sulfides. Several 20-mer ODNs including one with three dG and 17 dT were synthesized and subjected to cleavage and deprotection with DBU in different solvents including THF, ACN, DMF, DMSO and NMP at temperatures as high as 55 ^{o}C for up to 16 hours. No significant amount of ODN could be detected with RP HPLC. Using the stronger non-nucleophilic phosphazene base P2-Et[12] under similar conditions, no ODN was detected

either. Therefore, we were confident to conclude that Dmoc linker and protecting group have to be oxidized before they can be cleaved with non-nucleophilic bases such as potassium carbonate and DBU.

Sensitive ODN synthesis: In our previous studies, we have already demonstrated that a number of functional groups sensitive to cleavage and deprotection conditions used in known ODN synthesis methods such as concentrated ammonium hydroxide at elevated temperature and potassium carbonate in anhydrous methanol (i.e. dilute potassium methoxide) at room temperature can survive the conditions used for removing Dim and Dmoc groups and cleaving Dmoc linker. The sensitive groups tested included alkyl ester, aryl ester, thioester, alkyl halide, α-halo amide and chloropurine.[7, 9-11, 13] It should be reasonable to believe that these groups should survive the conditions used here for the deprotection of aDim and aDmoc groups because the conditions are the same. In the current study, we decided to test if N^4-acetyldeoxycytidine could survive the deprotection and cleavage conditions. N^4-Acetylcytidine (ac^4C) has been found in many RNAs including mRNA, tRNA and rRNA. It has important biological functions and is related to many human diseases.[14] If N^4-acetyldeoxycytidine could indeed survive the conditions involving sodium periodate and potassium carbonate, it would be easy to predict that the aDim-aDmoc ODN synthesis method could be extended to the synthesis of RNAs that contain the ac^4C nucleotide.

Accordingly, the 22-mer ODN (**29j**) was synthesized under the same conditions used for **29i**. The phosphoramidites **30a-d** were used. The N^4-acetyldeoxycytidine was incorporated with the commercially available **30f**, which is one of the most widely used phosphoramidites in standard ODN synthesis. Deprotection and cleavage were also conducted under the same conditions used for **29i**. No special attention was needed to prevent the loss of the highly sensitive acetyl group on the deoxycytidine nucleotide.[8] The ODN was purified with RP HPLC. The profiles of crude and pure ODN are in ESI. As expected, the ODN was easy to purify, and highly pure product can be readily obtained. MALDI MS analysis indicated that the acetyl group was not lost during the deprotection and cleavage process, and only molecular peaks including those with one to three charges were observed (Figure 4). To confirm the result, ODN **29k**, which has an identical sequence with **29j** but without the acetyl group, was obtained. A mixture of **29j** and **29k** was prepared and subjected to MALDI MS analysis. Molecular ions corresponding to both **29j** and **29k** were observed, and the difference of the two peaks matched well with the mass of an acetyl group (Figure 4). As expected, resolving **29j** and **29k** with other means such as RP HPLC and capillary electrophoresis were not easy. Under the conditions we used, analysis of the mixture gave a single peak in all trials.

Conclusions

In summary, aDim-aDmoc monomers (**5dNpe**, **5dNpr** and **5dNme**) were synthesized and studied for sensitive ODN synthesis. ODN deprotection were achieved under non-nucleophilic and nearly neutral conditions, which are required for sensitive ODN synthesis. Among them, the meDim-meDmoc monomers (i.e. **5dNme** monomers) are most ideal for sensitive ODN synthesis. In addition to be most atom economic, they are less hydrophobic than **5dNpe** and **5dNpr** monomers, and thus have less or no adverse effect on ODN synthesis. We also studied the CE-meDmoc phosphoramidites (**30a-c**). These phosphoramidites are easier to synthesize than **5dNme** phosphoramidites and can serve most purposes in sensitive ODN synthesis. Finally, we demonstrated that the highly sensitive N^4-acetyldeoxycytidine can survive the deprotection conditions used by the aDim-aDmoc methods. An ODN containing N^4-acetyldeoxycytidine was successfully synthesized and characterized with HPLC, capillary electrophoresis and MALDI MS. Improving the aDim-aDmoc technology to synthesize longer and more dG rich sequences as well as extending the technology for the synthesis of sensitive RNAs are in progress.

Experimental Section

ODN synthesis: ODNs **29a-k** were synthesized on dT-Dmoc-CPG (26 μmol/g loading, 20 mg, 0.52 μmol) using a MerMade 6 automated synthesizer. Detritylation: DCA (3% in DCM), 90 s × 2. Coupling: phosphoramidite (**5dNpe**, **5dNpr**, **5dNme**, **30a-d**, or **30f** 0.1 M in ACN), 4,5-dicyanoimidazole (0.25 M in ACN), 60 s × 3. Capping: 2-cyanoethyl *N*,*N*,*N*′,*N*′-tetraisopropylphosphoramidite (0.1 M, ACN), 4,5-dicyanoimidazole (0.25 M, ACN), 60 s × 3. Oxidation: I_2 (0.02 M, THF/pyridine/H_2O, 70/20/10, v/v/v), 40 s × 2. The last nucleotide at the 5'-end was incorporated using **13dNpe**, **13dNpr**, **13dNme** or **30e** under the same conditions as other synthetic cycles. The 5'-trityl group was kept.

ODN deprotection and cleavage: The CPG (0.52 μmol synthesis) was divided into five equal portions, and one portion was subjected to the following deprotection and cleavage conditions. DBU treatment: For ODNs **29i-j**, which were synthesized using **30a-f** and the ODNs can be represented with **32** (Scheme 6), to the CPG in a 1.5 mL centrifuge tube was added DBU/ACN (1/9, v/v, 1 mL). The mixture was shaken gently at rt for 15 min. The supernatant was removed with a pipette. The CPG was washed with ACN (1 mL × 5). This removed the 2-cyanoethyl protecting groups, and converted **32** to **33**. For ODNs **29a-h**, which were synthesized using **5dNpe**, **5dNpr**, **5dNme**, **13dNpe**, **13dNpr** or **13dNme** and the ODNs can be represented with **27** (Scheme 4), the DBU treatment is not needed. $NaIO_4$ oxidation: To the CPG (1/5 of the 0.52 μmol synthesis) represented by **27** or **33** in a 1.5 mL centrifuge tube, the solution of $NaIO_4$ in water (0.4 M, 1 mL), which has a pH of 4 without adding any acid, was added. The mixture was shaken gently at rt for 3 h. The supernatant was removed with a pipette, and the CPG was washed with water (1 mL × 5). In some instances, the oxidation step was repeated but this did not make much difference. This step converted **27** and **33** to **28** and **34**, respectively. K_2CO_3 treatment: To the CPG represented by **28** or **34** in a 1.5 mL centrifuge tube, K_2CO_3 (0.05%, pH 8, 1 mL) was added. The mixture was shaken gently at rt for 3 h. The supernatant was transferred into another 1.5 mL centrifuge tube. The CPG was washed with water (200 μL × 5). The supernatant and the washes were combined and concentrated to ~50 μL in a 1.5 mL centrifuge tube. *n*BuOH (450 μL) was added. The mixture was vortexed and then centrifuged (14.5k rpm, 15 min). The supernatant was carefully removed with a pipette without disturbing the ODN precipitate. This converted **28** and **34** to deprotected ODN **3** (Schemes 4 and 6). ODN **29k** was simply cleaved and deprotected by treating the CPG, which can be represented by **32**, with concentrated NH_4OH at 55 °C for 16 h.

ODN purification and analysis: The deprotected ODN (1/5 of the 0.52 μmol synthesis) was dissolved in water (100 μL). A portion of the solution (35 μL in the cases of **29a-f** and **29i-j**, 20 μL in the cases of **29g-h**) was injected into RP HPLC, which generated the profile of crude trityl-tagged ODN. Fractions of the peak corresponding to the full-length trityl-tagged ODN (retention time ~35-40 min) were collected and concentrated to ~100 μL. The solution was injected into HPLC, which generated the profile of pure trityl-tagged ODN. Fractions of the peak were collected, and concentrated to dryness. To the residue in a 1.5 mL centrifuge tube was added AcOH (80%, 1 mL). The tube was shaken gently at rt for 3 h. Volatiles were evaporated in a vacuum centrifugal evaporator. The residue was dissolved in water (100 μL) and injected into HPLC, which generated the profile of crude detritylated ODN. Fractions of the peak corresponding to the full-length detritylated ODN (retention time ~19 min) were collected and concentrated to dryness. To the residue in a 1.5 mL centrifuge tube was added water (100 μL). The solution was injected into HPLC, which generated the profile of pure detritylated ODN. Fractions of the ODN were collected and concentrated to dryness. The pure ODN was analyzed with MALDI MS and in selected cases capillary electrophoresis (CE). ODN **29k** was purified with trityl-on RP HPLC and analyzed with MALDI MS and CE as well. OD_{260} of all ODNs were determined using a

reported method.[15] HPLC profiles, MS images, CE profiles and OD_{260} values of all ODNs are in Figures 3-4 or ESI.

Electronic supplementary information (ESI) available: Experimental details, HPLC profiles, MALDI MS, capillary electrophoresis profiles, OD_{260} values of ODNs, and images of ^{1}H, ^{13}C and ^{31}P NMR spectra of compounds.

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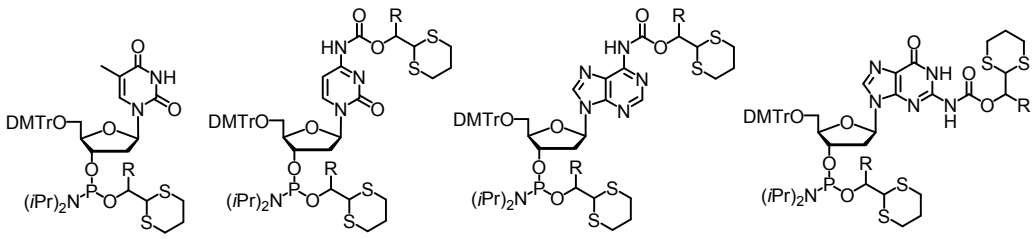
Figure 1:



5dTpe, R = Pe
5dTpr, R = Pr
5dTme, R = Me

5dCpe, R = Pe
5dCpr, R = Pr
5dCme, R = Me

5dApe, R = Pe
5dApr, R = Pr
5dAme, R = Me

5dGpe, R = Pe
5dGpr, R = Pr
5dGme, R = Me

Figure 1. aDim-aDmoc phosphoramidite monomers. The monomers are also called **5dNpe**, **5dNpr** and **5dNme**, wherein N is an abbreviation for nucleosides **T**, **C**, **A** and **G**.

Figure 2:

29a (11-mer), 5'-TTC TCT CTC TT-3'
29b (11-mer), 5'-TTA TAT ATA TT-3'
29c (11-mer), 5'-TTA TGT ATA TT-3'
29d (10-mer), 5'-TCT ATC TCT T-3'
29e (19-mer), 5'-TTT AGT CAT CTT CTT TTC T-3'
29f (19-mer), 5'-TCG TAC CAT CTT TAA ACA T-3'
29g (20-mer), 5'-TTT TTC CAT CCT AGA AAG CT-3'
29h (23-mer), 5'-TCA CAT TAT ACC ATT CTC CTA AT-3'
29i (19-mer), 5'-TAG TAC TTT ATC CAA CCT T-3'
29j (22 Mer), 5'-TCA TAG TA**(ac)C** TTT ATC CAA CCT T-3'
29k (22 Mer), 5'-TCA TAG TA**C** TTT ATC CAA CCT T-3'

Figure 2. ODN sequences. ODNs **29a**-**c**, **29d**-**f**, **29g**-**h**, and **29i**-**k** were synthesized using phosphoramidites **5dNpe**, **5dNpr**, **5dNme**, **30a**-**d**, respectively. All were deprotected and cleaved under non-nucleophilic and nearly neutral conditions except for **29k**, which was deprotected and cleaved using traditional conditions involving concentrated ammonium hydroxide at elevated temperature.

Figure 3:

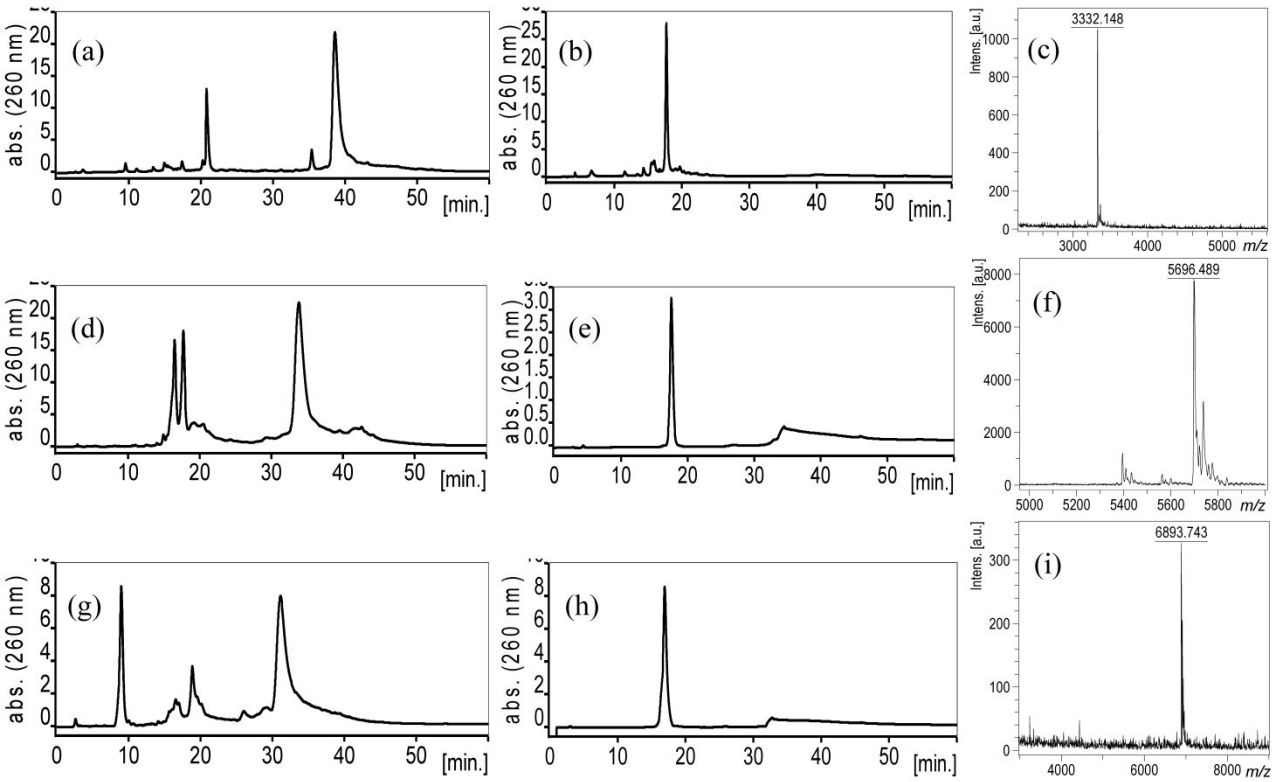


Figure 3. RP HPLC profiles and MALDI MS of ODNs synthesized using the aDim-aDmoc technology. (a) HPLC of crude ODN **29c**. (b) HPLC of crude ODN **29c** from detritylation of RP HPLC purified trityl-on ODN. (c) MS of pure ODN **29c**, calcd for $[M-H]^-$ 3333.6 found 3332.1. (d) HPLC of crude ODN **29e**. (e) HPLC of pure ODN **29e**. (f) MS of pure ODN **29e**, calcd for $[M-H]^-$ 5696.9 found 5696.5. (g) HPLC of crude ODN **29h**. (h) HPLC of pure ODN **29h**. (i) MS of pure ODN **29h**, calcd for $[M-H]^-$ 6888.2 found 6893.7.

Figure 4:

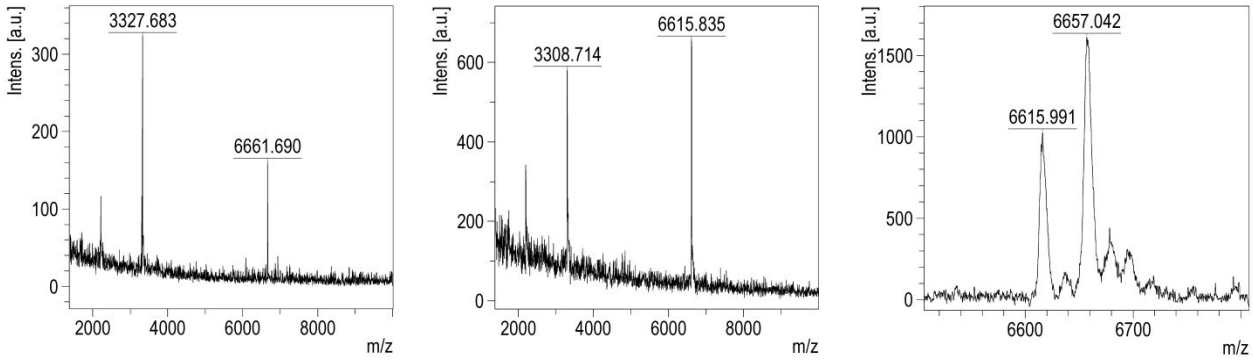
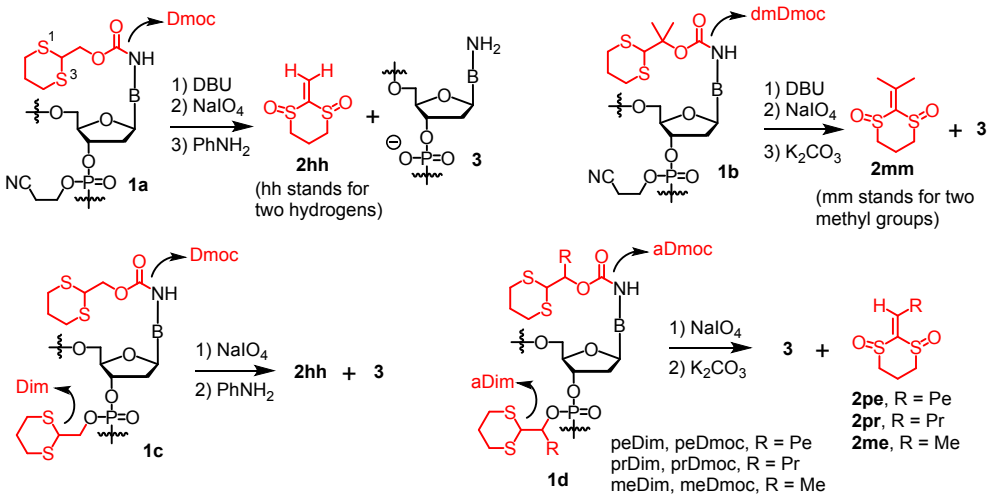


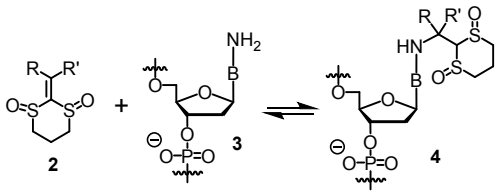
Figure 4. MALDI MS of the 22-mer ODN **29j** that contains the sensitive N^4-acetyldeoxycytidine nucleoside. The MS of **29k**, which has the same sequence as **29j** but lack the acetyl group, is also included. Left: MS of **29j**, calcd for $[M+H]^+$ 6659.1 found 6661.7. Middle: MS of **29k**, calcd for $[M-H]^-$ 6615.1 found 6615.8. Right: mixture of **29j** and **29k**, calcd for **29j** $[M-H]^-$ 6657.1 and **29k** $[M-H]^-$ 6615.1, found **29j** 6657.0 and **29k** 6616.0. The mass difference 41.0 between **29j** and **29k** matches the mass 42.0 of $CH_3CO - H$.

Scheme 1:



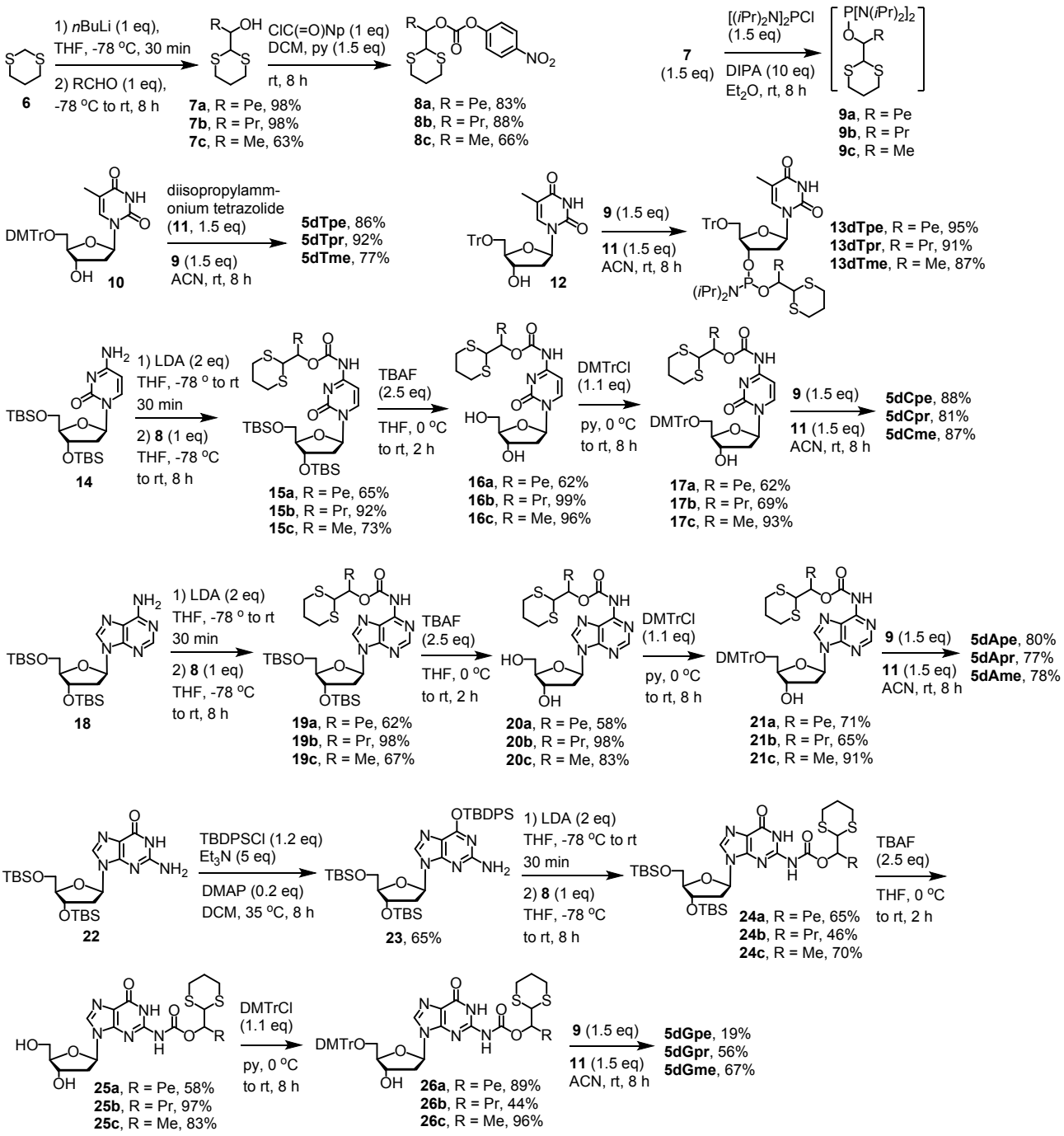
Scheme 1. Summary of deprotection conditions and deprotection side products for ODNs synthesized using Dmoc, dmDmoc, Dim-Dmoc and aDim-aDmoc as protecting groups.

Scheme 2:



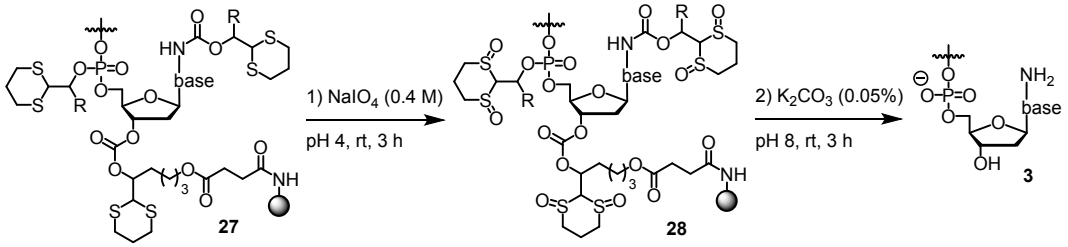
Scheme 2. Michael addition side reaction between deprotection side product **2** and deprotected ODN **3**.

Scheme 3:



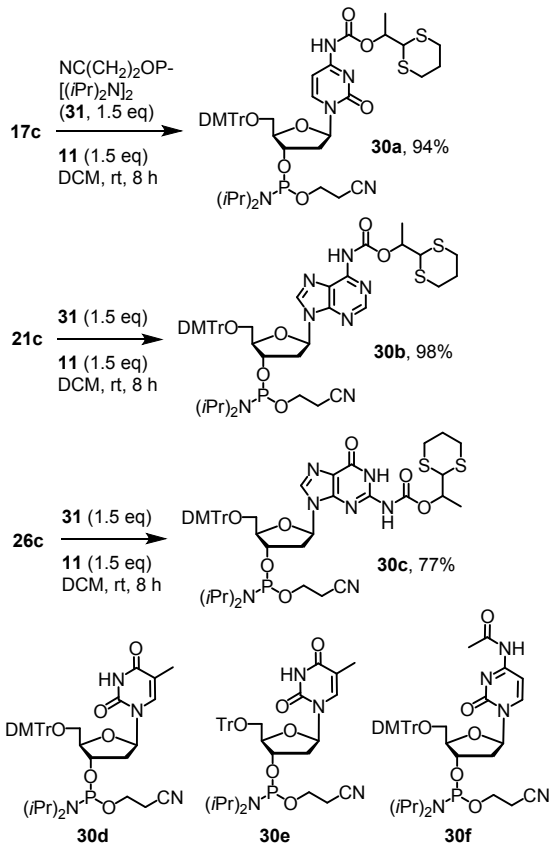
Scheme 3. Synthesis of aDim-aDmoc phosphoramidite monomers.

Scheme 4:



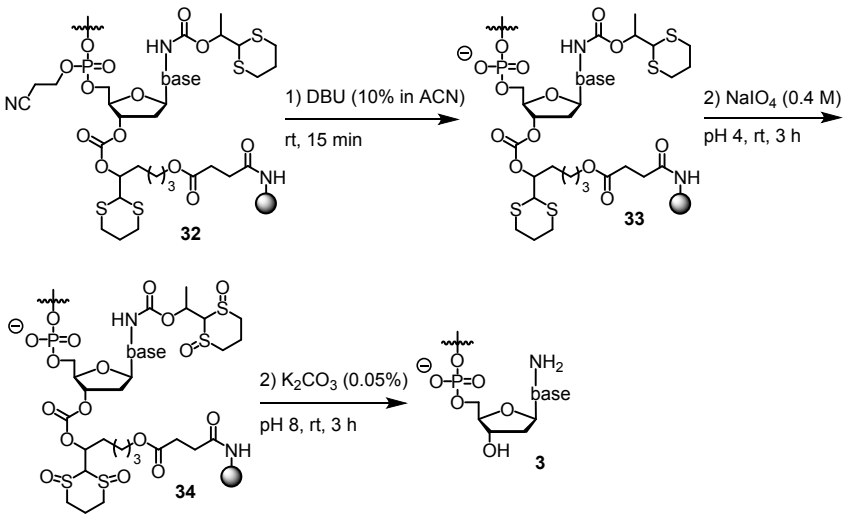
Scheme 4. Deprotection and cleavage of ODNs synthesized using aDim-aDmoc protected phosphoramidites.

Scheme 5:



Scheme 5. Synthesis of CE-meDmoc phosphoramidites.

Scheme 6:



Scheme 6. Deprotection and cleavage of ODNs synthesized using CE-meDmoc protected phosphoramidites.