Organic & Biomolecular Chemistry

PEGylated Dmoc Phosphoramidites for Sensitive Oligodeoxynucleotide Synthesis

Journal:	*Organic & Biomolecular Chemistry*
Manuscript ID	OB-ART-09-2023-001495.R1
Article Type:	Paper
Date Submitted by the Author:	28-Oct-2023
Complete List of Authors:	Chillar, Komal; Michigan Technological University, Department of Chemistry and Health Research Institute Yin, Yipeng; Michigan Technological University, Department of Chemistry and Health Research Institute Apostle, Alexander ; Michigan Technological University, Department of Chemistry and Health Research Institute Fang, Shiyue; Michigan Technological University, Department of Chemistry and Health Research Institute

ARTICLE

PEGylated Dmoc Phosphoramidites for Sensitive Oligodeoxynucleotide Synthesis

Komal Chillar,[a] Yipeng Yin,[a] Alexander Apostle[a] and Shiyue Fang*[a]

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

Abstract: Sensitive oligodeoxynucleotides (ODNs) can be synthesized using Dmoc phosphoramidites, but only short ODNs were demonstrated. Here, we report the synthesis of much longer ODNs, which was made possible by the use of PEGylated Dmoc (pDmoc) phosphoramidites. The longer ODNs synthesized include those containing the sensitive 4acC epigenetic modification recently discovered in nature.

Introduction

Over one hundred epigenetically modified nucleotides have been found in DNA and RNA.[1] These modifications serve as an additional layer of regulation in the biological system. Malfunction of this layer of regulation has been found to be related to many human diseases.[2] Among the modifications, many are sensitive to nucleophiles. For example, N^4-acetylcytosine (ac4C) in RNA[3] and the recently detected N^4-acetyldeoxycytosine (4acC) in DNA[4] cannot survive nucleophilic conditions such as those involving dilute potassium methoxide or ammonia. Standard DNA and RNA synthesis methods use saturated ammonium hydroxide at elevated temperature for deprotection and cleavage, and therefore are unsuitable for the synthesis of oligonucleotides containing 4acC or ac4C.[5] Some efforts have been made to address the problem,[6] but a practical solution is lacking. For example, Sekine, etc. were able to synthesize 4acC containing ODNs, but all other nucleotides in the sequences were dT.[6e] Meier, etc. reported the synthesis of ac4C containing RNAs, but unprotected G phosphoramidite, which does not allow capping failure sequences, was used.[6f] To generate ac4C antibody, Meier, etc. prepared ac4C containing RNAs using *in vitro* transcription, but the method lacks efficiency and specificity.[6g]

In 2016, we reported the use of the 1,3-dithian-2-yl-methoxycarbonyl (Dmoc) function as protection groups and linker for oligodeoxynucleotide (ODN) synthesis.[7] Deprotection and cleavage were achieved under non-nucleophilic conditions involving oxidation with sodium periodate at pH 4 followed by *β*-elimination at pH 8. Subsequently, significant efforts have been devoted to improve the method, and various sensitive groups have been demonstrated to be able to survive the deprotection and cleavage conditions.[8] However, the longest ODN that could be synthesized is a 23-mer.[9] In this paper, we report the use of PEGylated Dmoc (pDmoc) phosphoramidites for sensitive ODN synthesis. Our hypothesis was that the limited length of ODNs that could be synthesized using the Dmoc methods was due to the hydrophobicity of the Dmoc group. When the ODNs on the solid support reached certain length, the cumulative hydrophobic effect of Dmoc groups reduced the solubility of ODN, and therefore, subsequent reactions became less efficient. Because PEGylation can increase solubility of materials, we decided to test if PEGylated Dmoc (pDmoc) phosphoramidites (Figure 1) could address the problem. Indeed, using pDmoc phosphoramidites, we were able to synthesize ODNs with length up to 49 nucleotides. In addition, to demonstrate the method for sensitive ODN synthesis, ODN sequences selected

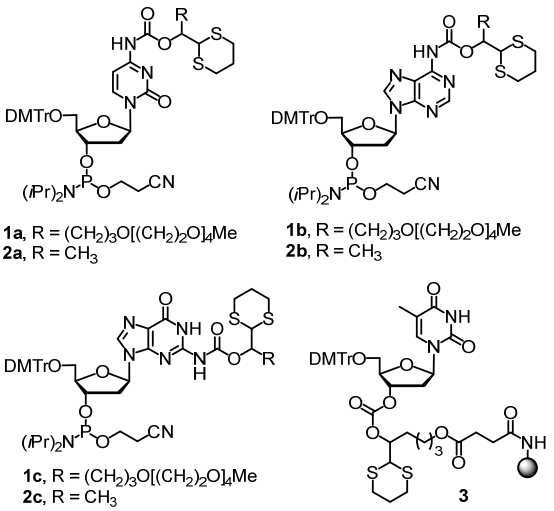
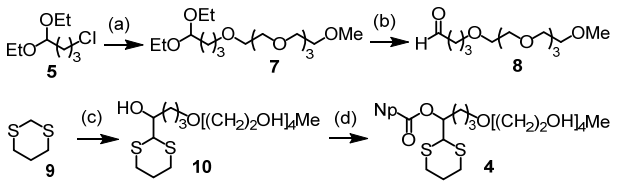


Figure 1. pDmoc and meDmoc phosphoramidites and Dmoc linker.

[a] *Department of Chemistry and Health Research Institute, Michigan Technological University, 1400 Townsend Drive, Houghton, MI 49931, USA. E-mail: shifang@mtu.edu*

† Electronic Supplementary Information (ESI) available: Experimental details; HPLC, MALDI MS, OD_{260}, capillary electrophoresis, and trityl assay data for ODNs; and NMR spectra of new compounds. See DOI: 10.1039/x0xx00000x

from the genomic DNA of *Arabidopsis* containing two 4acC groups have been synthesized.[4] All the ODNs were characterized with HPLC, MALDI MS and capillary electrophoresis.



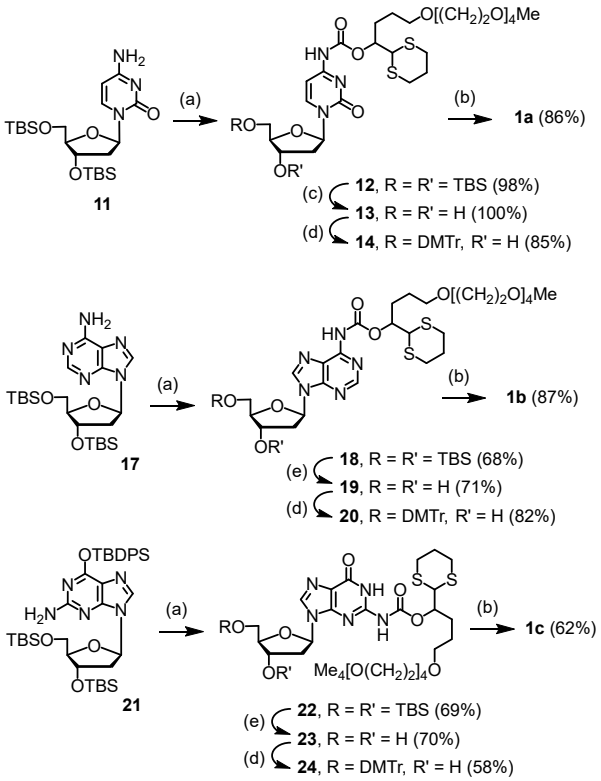
Scheme 1. Synthesis of reagent (**4**) for installing pDmoc. (a) $HO[(CH_2)_2O]_4Me$ (**6**, 1 eq), NaH (1.5 eq), THF, rt to 50 °C, 8 h. (b) HCl (5%), rt, 3 h, 85% from **5**. (c) *n*BuLi (1 eq), THF, -78 °C, 30 min, then **8** (1 eq), -78 °C to rt, 8 h, 92%. (d) ClC(=O)Np (1 eq), DCM, pyridine (1.5 eq), rt, 8 h, 87%. Np, 4-nitrophenol.

Results and Discussion

Synthesis of pDmoc phosphoramidites

Our plan was to use pDmoc phosphoramidites **1a**-**c** (Figure 1) to increase the solubility of the growing ODN and thus improve the efficiency of reactions for ODN synthesis. The majority of the monomers for the synthesis would still be the non-PEGylated monomers **2a**-**c** as well as the commercial 5'-DMTr-dT-CE phosphoramidite. Monomers **1a**-**c** would only be used occasionally because they are less atom economic. The meDmoc group was chosen over Dmoc because its deprotection does not need a scavenger.[8c, 9] All the syntheses were planned to be carried out using linker **3**, which allows ODN cleavage under the same non-nucleophilic conditions for Dmoc deprotection. Thus, the plan required monomers **1a**-**c**, **2a**-**c** and linker **3** (Figure 1). The synthesis of **2a**-**c** and **3** has been reported earlier.[7, 9] For the synthesis of **1a**-**c**, the PEGylation agent **4** was needed (Scheme 1). Commercial **5** was reacted with $HO(PEG)_4OMe$ (**6**) to give **7**, which upon treating with an acid gave **8**. Reaction of **8** with deprotonated **9** gave **10**. Compound **10** was converted to **4** by reacting with *p*-nitrophenyl chloroformate. All the reactions were simple, and the yields were high (ESI).

For the synthesis of **1a** (Scheme 2), cytidine nucleoside derivative **11**[9] was treated with 2 equivalents LDA and 1 equivalent **4** to give the pDmoc protected dC **12**. This condition for the introduction of carbamate groups to arylamines was known.[10] The TBS groups of **12** was removed to give **13**, the 5'-OH was protected with DMTr-Cl to give **14**. Compound **14** was phosphitylated to give the target pDomc dC phosphoramidite **1a** under standard conditions.[11] The pDmoc dA phosphoramidite **1b** was synthesized under similar conditions. For the synthesis of pDmoc dG phosphoramidite **1c**, the procedure was similar except that **21**, of which the lactam of the nucleobase was protected with a TBDPS group,[9] was used as the starting material. Good to excellent yields were obtained for the steps for the synthesis of **1a**-**b**. For **1c**, the yields were slightly lower, but they were still acceptable. We had no difficulty in obtaining sufficient amount of the materials for ODN synthesis.



Scheme 2. Synthesis of pDmoc phosphoramidites. (a) LDA (2 eq), THF, -78 °C to rt, 30 min; then **4** (1 eq), THF, -78 °C, to rt, 8 h. (b) $NC(CH_2)_2OP[N(iPr)_2]_2$, (**15**, 1.5 eq), diisopropylammonium tetrazolide (**16**, 1.5 eq) DCM, rt, 8 h. (c) TBAF, THF, rt, 2 h. (d) DMTr-Cl, pyridine, rt, 8 h. (e) Et_3N-3HF, THF, rt, 2 h.

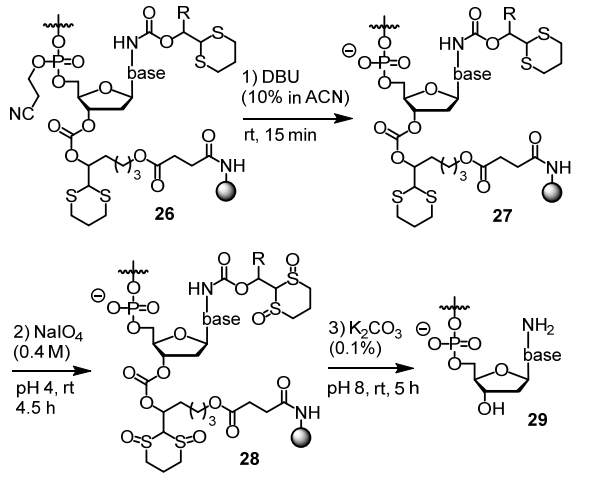
Longer ODN synthesis using pDmoc phosphoramidites

With monomers **1a**-**c**, **2a**-**c** and linker **3**, we started to test the idea of using PEGylation to increase the length of ODN that can be synthesized. The 30-mer **25a** (Figure 2), which does not contain any sensitive group, was used for the initial study. Without using the PEGylation strategy, the longest ODN that were synthesized was a 23-mer.[9] The current synthesis was carried out under standard conditions with the following modifications. For the coupling step, the majority of the nucleotides dA, dC and dG in the sequence were incorporated with a meDmoc phosphoramidite (**2a**-**c**), but for the incorporation of roughly every other five nucleotides, the pDmoc phosphoramidite (**1a**-**c**) was used (see Figure 2). As stated earlier, the PEG moieties introduced to the ODN was intended to increase ODN solubility. According to trityl assay (see ESI), the coupling efficiency was comparable with standard phosphoramidites. Considering the facts that a 30-mer synthesis with 99% coupling efficiency only gives theoretically 74% full-length ODN and besides truncated sequences,

25a 30-mer: TAAC<u>T</u>TTAT<u>C</u>GTA<u>CC</u>AT<u>C</u>TTTAAA<u>C</u>ATATT
25b 30-mer: T<u>G</u>TCCTT<u>A</u>CCTTC<u>A</u>TTCC<u>G</u>TTC<u>A</u>TCTT
25c 38-mer: TGGA<u>C</u>TTGTAA<u>C</u>TTTAT<u>C</u>GTA<u>CC</u>AT<u>C</u>TTTAAA<u>C</u>ATATT
25d 49-mer: TA<u>CC</u>GAATGGATGGA<u>C</u>TTGTAA<u>C</u>TTTAT<u>C</u>GTA<u>CC</u>A
T<u>C</u>TTTAAA<u>C</u>ATATT
25e 28-mer: TA<u>C</u>TAGTA<u>C</u>**TC(Ac)**TT<u>C</u>TT**C(Ac)**TTCTT<u>C</u>TTCTT
25f 29-mer: T<u>C</u>TTAT<u>C</u>**TC(Ac)**TC<u>C</u>**TC(Ac)**TTTTTTGG<u>CC</u>TTTT
25g 29-mer: T<u>C</u>GAAA<u>C</u>GC**C(Ac)**AT<u>C</u>TCCG**C(Ac)**<u>C</u>GTTAAT<u>C</u>TCT
25h 28-mer: TACTAGTACT **C**TTCTT**C**TTCTTCTTCTT
25i 29-mer: TCTTATCT**C**TCTCT**C**TTTTTTGGCCTTTT
25j 29-mer: TCGAAACGC**C**ATCTCCG**C**CGTTAATCTCT

Figure 2. ODN sequences. For **25a**-**g**, nucleotides underlined were incorporated using pDmoc phosphoramidites (**1a**-**c**); those not underlined were incorporated using Dmoc (**2a**-**c**), or standard dT or dC^{Ac} [for C(Ac)] phosphoramidites. For **25h**-**j**, all nucleotides were incorporated using standard phosphoramidites. ODNs **25e**-**g** contain two 4acC each. The sequences of **25h**-**j** are identical with **25e**-**g**, respectively, except that 4acC are replaced by dC (bold).

the crude ODN contains other impurities such as small molecules and pre-detritylated full-length ODN, the synthesis yields indicated by crude HPLC profiles (see ESI) are not inconsistent with results of trityl assay. For capping, instead of acetic anhydride, 2-cyanoethyl *N*,*N*,*N*′,*N*′-tetraisopropylphosphoramidite and 4,5-dicyanoimidazole (DCI) were employed. This was intended to prevent cap-exchange. For the last synthetic cycle, a 5'-trityl (Tr) instead of 5'-DMTr phosphoramidite was used for the coupling step. The reason is that the DMTr group is not stable under the sodium periodate oxidation conditions (pH 4) used during ODN deprotection. The Tr group is stable, and can assist RP HPLC purification of the ODN product. At the end of the ODN synthesis, the product can be represented as **26** (Scheme 3), in which the ODN is anchored to the CPG via a Dmoc linker, the phosphate is protected with a 2-cyanoethyl group, and the *exo*-amino groups are protected with Dmoc or pDmoc group.



Scheme 3. ODN deprotection and cleavage.

ODN deprotection and cleavage were carried out under the same conditions used previously when no pDmoc phosphoramidites were used.[9] Briefly, the CPG (**26**, Scheme 3) was treated with DBU to remove the 2-cyanoethyl groups giving **27**. The Dmoc groups introduced by meDmoc and pDmoc phosphoramidites were oxidized with 0.4 M sodium periodate, which is slightly acidic (pH 4) by itself, to give **28**. At this stage, the ODN was still on the solid support, which is important because it allows easy separation of sodium periodate and its reduced product from the ODN by washing with water. Finally, treating **28** with dilute potassium carbonate cleaved the ODN from the solid support and gave the fully deprotected ODN **29**.

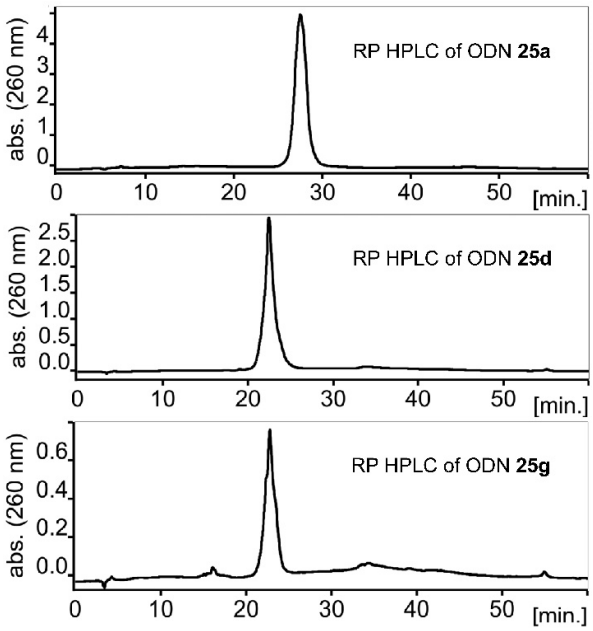


Figure 3. RP HPLC of ODNs. See ESI for detailed conditions for HPLC.

The ODN (**25a**) was purified with Tr-on RP HPLC. The trityl group was removed with 80% acetic acid. RP HPLC showed that the ODN was pure (Figure 3). The purified ODN was analyzed with MALDI MS. Correct molecular peak was found (Figure 4). The purity of the ODN was further analyzed with capillary electrophoresis (CE, see ESI). Because previously without using pDmoc phosphoramidites, we were not able to synthesize ODNs longer than 23-mers, our hypothesis that PEGylation can increase ODN solubility and enable longer ODN synthesis is validated. Encouraged by the results, we made efforts to synthesize different and longer ODNs. Under the same conditions for the synthesis of **25a**, the 30-mer **25b**, 38-mer **25c** and the 49-mer **25d** (Figure 2) were successfully synthesized. The HPLC profile and MALDI MS of **25d** are shown in Figures 3 and 4, respectively. Additional purification and analysis data are in ESI.

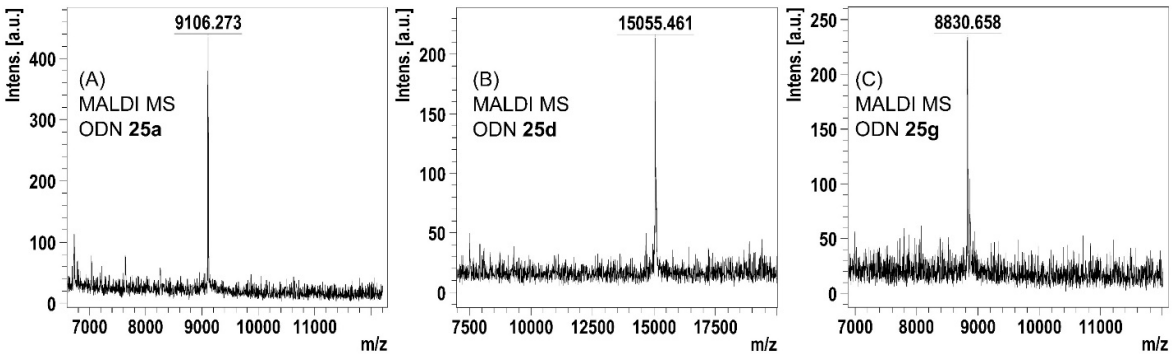


Figure 4. MALDI MS of ODNs. (A) MS of **25a**. Calcd for $[M+NH_4]^+$ *m/z* 9105, found 9106. (B) MS of **25d**. Calcd for $[M+K]^+$ *m/z* 15054, found 15055. (C) MS of **25g**. Calcd for $[M-H]^-$ *m/z* 8831, found 8831.

To see if the method can synthesize even longer ODNs, we briefly tested incorporating all nucleotides with pDmoc (and dT) phosphoramidites. The results were unsatisfactory. Once 20-mer was reached, the synthesis became ineffective as indicated by trityl assay. The large mass introduced by PEGs could be the cause. Evidently, to synthesize longer ODNs, some engineering work for determining the number and location of pDmoc phosphoramidites that should be used in specific sequences is required.

Sensitive ODN synthesis using pDmoc phosphoramidites

Recently Wu's group reported the detection of 4acC in genomic DNAs of *Arabidopsis*, rice, maize, mouse and human, and potential biological roles of the epigenetic modification.[4] Previously, ac4C was known in RNAs, and significant efforts have made to study its biological functions and relations to human diseases.[3b, 12] However, until the recent report, there was no information about 4acC in DNAs in nature. Motivated by the report, we synthesized ODNs **25e**-**g** (Figure 2). The sequences were from the regions of chromosome 1 of *Arabidopsis*, in which 4acC modifications were detected.[4] They contain the 4acC motifs CDYCDYCDYCDYCDY or YCTCTCTYTCTYYYT (D represents A/G/T; Y represents C/T), and thus chances exist that they may carry 4acC in cells. The motifs are similar to those of many transcription factors suggesting that 4acC may play a role in the regulation of gene expression.[4] To challenge our method, two 4acC modifications instead of one were placed in each ODN. It is notable that using the Dmoc-pDmoc method, no special manipulations are needed. The HPLC profile and MALDI MS of the 29-mer ODN **25g** are shown in Figures 3 and 4, respectively. All data regarding purification and analysis of **25e**-**g** are in ESI. The presence of the two acetyl groups of 4acC in the sequences was further confirmed by comparing their MALDI MS with those of ODNs **25h**-**j**, which have identical sequences with **25e**-**g**, respectively, but do not contain acetyl group. As shown in Figure 5, the difference of the molecular peaks for **25g** and **25j** matches closely with the mass of two acetyl groups. It is noted that the peak at *m/z* 8787 may also be from the molecule corresponding to **25g** with one acetyl group lost. However, we believe that this is unlikely because this peak is absent in the MS spectrum of **25g** without intentionally added **25j** (Figure 4C and ESI). The data regarding **25e**-**f** are provided in ESI.

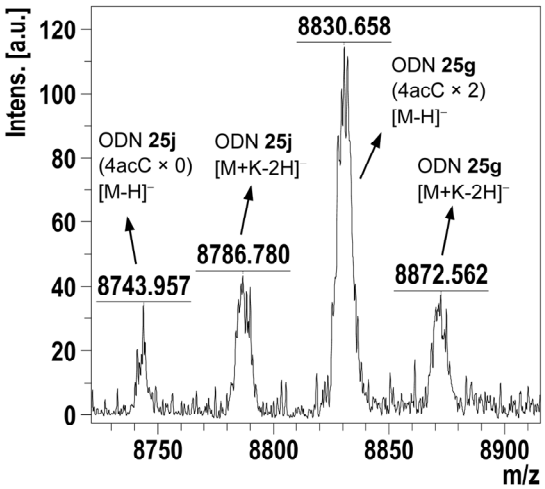


Figure 5. MALDI MS of the mixture of ODNs **25g** and **25j**. Calcd for **25g** $[M-H]^-$ 8830, found 8831; $[M-2H+K]^-$ 8869, found 8873. Calcd for **25j** $[M-H]^-$ 8746, found 8744; $[M-2H+K]^-$ 8782, found 8787. The mass difference between the molecular peaks for the two ODNs (87) is close to the mass of two acetyl groups minus two hydrogen (84).

The use of PEGylation to increase the length of ODNs is notable. To our knowledge, for solid phase ODN synthesis, the cause of inability to synthesize longer ODNs has always been attributed to the cumulative effect of non-quantitative stepwise yields. In an unrelated study, we found that even though DMSO is miscible with many solvents, the solubility of oligosulfoxides decreases rapidly as their lengths increase.[13] This prompted us to hypothesize that as ODNs grow, their solubility may decrease, and the reactions may become less efficient. Our success in the present study may inspire the use of PEGylation

to improve the efficiency of unmodified DNA and RNA synthesis, which can have a high impact on projects in areas such as synthetic biology,[14] protein engineering,[15] mRNA vaccine development[16] and DNA computer digital data storage[17] in which the availability of long DNA and RNA is crucial for success. In addition, the synthesis of ODNs containing more than one 4acC modification is remarkable. The acetyl group of 4acC and ac4C is notably labile. The present method is expected to be able to provide sensitive ODNs with sufficient length and number of sensitive groups that are practically useful for biological studies. For example, ODNs containing one or more 4acC modifications can help to identify proteins that interact with 4acC. Such proteins include 4acC writers, readers and erasers. The ODNs can also be used for studying the effects of acetylation of dC nucleotide on cellular stability of DNA, higher order structures of DNA, and efficiency of transcription.[3, 18]

Conclusions

In summary, using Dmoc as protecting group for ODN synthesis, deprotection can be achieved under non-nucleophilic conditions. As a result, the Dmoc method is useful for the synthesis of epigenetically modified ODNs containing functions sensitive to nucleophiles. However, the hydrophobicity of the Dmoc groups limited the length of ODNs that can be synthesized. Using pDmoc phosphoramidites, due to the increased solubility of the protected ODNs on the solid support, in the present work, synthesis of ODNs with up to 49 nucleotides have been achieved. To demonstrate the application of the method for sensitive ODN synthesis, three ODN sequences selected from the regions of the *Arabidopsis* genome that were found to have the 4acC modification have been successfully synthesized. All the ODNs were characterized with RP HPLC, MALDI MS and CE. The availability of a method for the synthesis of ODNs with suitable length and multiple sensitive groups is expected to be helpful for projects in the research area of epigenetics.

Experimental

ODNs were synthesized on an MerMade 6 synthesizer on dT-Dmoc-CPG (**3**, 26 μmol/g loading, 20 mg, 0.52 μmol) using the phosphoramidite chemistry. Deblocking: DCA (3%, DCM), 90 sec × 2. Coupling: Phosphoramidite (**1a**-**c**, **2a**-**c**, 5'-DMTr-dT-CE phosphoramidite, 5'-Tr-dT-CE phosphoramidite for last synthesis cycle, 0.1 M, MeCN), 4,5-dicyanoimidazole (DCI, 0.25 M, MeCN), 60 sec × 3. Capping: 2-Cyanoethyl *N*,*N*,*N*′,*N*′-tetraisopropylphosphorodiamidite (0.1 M, MeCN), DCI (0.25 M, MeCN), 60 sec × 3. Oxidation: I_2 (0.02 M, THF/pyridine/H_2O, 70:20:10, v/v/v), 40 sec × 2. At the end of the synthesis, the 5'-Tr group was kept. The CPG (**26**) was divided into 5 equal portions (~0.104 μmol each). One portion was subject to deprotection and cleavage. Removing 2-cyanoethyl groups: The suspension of CPG (**26**, ~0.104 μmol ODN) in the solution of DBU in anhydrous MeCN (1:9, v/v, 1 mL) in a 1.5 mL centrifuge tube was gently shaken at rt for 5 min. The supernatant was removed. The process was repeated two more times. The CPG was washed with anhydrous MeCN (1 mL × 5). Oxidation of Dmoc groups: The suspension of CPG (**27**) in the solution of $NaIO_4$ (0.4 M, 1 mL), which has a pH of 4, in a 1.5 mL centrifuge tube was gently shaken at rt for 1.5 h. The supernatant was removed. The process was repeated two more times. The CPG was washed with water (1 mL × 5). Removing oxidized Dmoc groups: The suspension of CPG (**28**) in the solution of K_2CO_3 (0.1%, 1 mL), which has a pH of 8, in a 1.5 mL centrifuge tube was gently shaken at rt for 5 h. The supernatant was transferred into a clean 1.5 mL centrifuge tube. The CPG was washed with water (150 μL × 5). The combined supernatant and washes were concentrated to ~50 μL. To the solution was added *n*BuOH (450 μL). After mixing by vortex, ODN was precipitated via centrifugation (14.5k rpm, ~14k × g, 15 min). The supernatant was removed leaving deprotected ODN (**29**) in the tube. RP HPLC purification: ODN (**29**) was dissolved in H_2O (100 μL). A portion (35 μL) was injected into HPLC to generate the crude ODN profile. Fractions under the major ODN peak at ~35 min were collected, and concentrated to ~100 μL, which was re-injected into HPLC to obtain the profile of pure trityl-tagged ODN. The fractions of the ODN were collected and concentrated to dryness. To the trityl-tagged ODN was added AcOH (80%, 1 mL). The mixture was shaken gently at rt for 3 h. Volatiles were evaporated. The residue was dissolved in water (100 μL) and injected into HPLC to generate the profile of crude de-tritylated ODN. The fractions of the ODN were collected and concentrated to dryness. The fractions of ODN at ~20 min was collected and concentrated to dryness. The residue was dissolved in water (100 μL) and injected into HPLC to generate the profile of pure de-tritylated ODN. The fractions of ODN were collected and concentrated to dryness. ODNs were quantified using a reported method,[19] and analyzed using MALDI-TOF MS and capillary electrophoresis.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

Financial support from NIH (GM109288), NSF (1954041), Robert and Kathleen Lane Endowed Fellowship (K.C., Y.Y., A.A.), Fleming-Skochelak Fellowship (K.C.) and HRI Fellowship (A.A.); assistance from D.W. Seppala (electronics), J.L. Lutz (NMR) and A. Galerneau (MS); and NSF equipment grants (2117318, NMR; 1048655 & 1531454, MS); are gratefully acknowledged.

Notes and references

1 T. Carell, C. Brandmayr, A. Hienzsch, M. Muller, D. Pearson, V. Reiter, I. Thoma, P. Thumbs and M. Wagner, *Angew. Chem. Int. Ed.*, 2012, **51**, 7110-7131.

2 (a) Y.G. Pei and E.S. Robertson, *Viruses-Basel*, 2020, **12**, doi:10.3390/v12121377; (b) E.S. Oh and A. Petronis, *Nat. Rev. Genet.*, 2021, **22**, 533-546.

3 (a) R. Karthiya, S.M. Wasil and P. Khandelia, *Mol. Biol. Rep.*, 2020, **47**, 9189-9199; (b) G. Jin, M. Xu, M. Zou and S. Duan, *Mol. Ther. Nucleic Acids*, 2020, **20**, 13-24.

4 S. Wang, H. Xie, F. Mao, H. Wang, S. Wang, Z. Chen, Y. Zhang, Z. Xu, J. Xing, Z. Cui, X. Gao, H. Jin, J. Hua, B. Xiong and Y. Wu, *Genome Biol.*, 2022, **23**, doi:10.1186/s13059-13021-02578-13057.
5 G. Meher, N.K. Meher and R.P. Iyer, *Curr. Protoc. Nucleic Acid Chem.*, 2017, **69**, doi:10.1002/cpnc.1032.
6 (a) J.C. Schulhof, D. Molko and R. Teoule, *Tetrahedron Lett.*, 1987, **28**, 51-54; (b) T.J. Matray and M.M. Greenberg, *J. Am. Chem. Soc.*, 1994, **116**, 6931-6932; (c) Y. Hayakawa, S. Wakabayashi, H. Kato and R. Noyori, *J. Am. Chem. Soc.*, 1990, **112**, 1691-1696; (d) R. Eritja, J. Robles, A. Avino, F. Albericio and E. Pedroso, *Tetrahedron*, 1992, **48**, 4171-4182; (e) T. Wada, A. Kobori, S.I. Kawahara and N. Sekine, *Eur. J. Org. Chem.*, 2001, **2001**, 4583-4593; (f) D. Bartee, K.D. Nance and J.L. Meier, *J. Am. Chem. Soc.*, 2022, **144**, 3487-3496; (g) W.R. Sinclair, D. Arango, J.H. Shrimp, T.T. Zengeya, J.M. Thomas, D.C. Montgomery, S.D. Fox, T. Andresson, S. Oberdoerffer and J.L. Meier, *ACS Chem. Biol.*, 2017, **12**, 2922-2926.
7 X. Lin, J. Chen, S. Shahsavari, N. Green, D. Goyal and S. Fang, *Org. Lett.*, 2016, **18**, 3870-3873.
8 (a) S. Shahsavari, D. Eriyagama, J. Chen, B. Halami, Y. Yin, K. Chillar and S. Fang, *J. Org. Chem.*, 2019, **84**, 13374-13383; (b) S. Fang, D. Eriyagama, Y. Yuan, S. Shahsavari, J. Chen, X. Lin and B. Halami, *Curr. Protoc. Nucleic Acid Chem.*, 2020, **82**, doi:10.1002/cpnc.1111; (c) S. Shahsavari, D. Eriyagama, B. Halami, V. Begoyan, M. Tanasova, J. Chen and S. Fang, *Beilstein J. Org. Chem.*, 2019, **15**, 1116-1128; (d) B. Halami, S. Shahsavari, Z. Nelson, L. Prehoda, D.N.A.M. Eriyagama and S.Y. Fang, *ChemistrySelect*, 2018, **3**, 8857-8862.
9 K. Chillar, A.M.D.N. Eriyagama, Y.P. Yin, S. Shahsavari, B. Halami, A. Apostle and S.Y. Fang, *New. J. Chem.*, 2023, **47**, 8714-8722.
10 (a) S. Shahsavari, J. Gooding, T. Wigstrom and S. Fang, *ChemistrySelect*, 2017, **2**, 3959-3963; (b) S. Shahsavari, C. McNamara, M. Sylvester, E. Bromley, S. Joslin, B.Y. Lu and S.Y. Fang, *Beilstein J. Org. Chem.*, 2018, **14**, 1750-1757.
11 D. Pokharel and S.Y. Fang, *Green Chem.*, 2016, **18**, 1125-1136.
12 (a) Y.Z. Ma, W.Z. Li, C. Fan, Y.Z. Wang, H. Jiang and W.M. Yang, *J. Alzheimers Dis.*, 2022, **90**, 1659-1675; (b) R. Karthiya, S.M. Wasil and P. Khandelia, *Mol. Biol. Rep.*, 2020, **47**, 9189-9199.
13 B. Halami, D.N.A.M. Eriyagama, K. Chillar, Z. Nelson, L. Prehoda, Y.P. Yin, B.Y. Lu, B. Otto, L. Haggerty and S.Y. Fang, *Tetrahedron Lett.*, 2019, **60**, doi:10.1016/j.tetlet.2019.151306.
14 K.L. Garner, *Essays Biochem*, 2021, **65**, 791-811.
15 M. Chai, X.H. Xu, Y.F. Liu, J.H. Li, G.C. Du, X.Q. Lv and L. Liu, *ACS Synth. Biol.*, 2023, doi:10.1021/acssynbio.1023c00026.
16 A.A.A. Aljabali, R.M. Bashatwah, M.A. Obeid, V. Mishra, Y. Mishra, A. Serrano-Aroca, K. Lundstrom and M.M. Tambuwala, *Drug Discov. Today*, 2023, **28**, doi:10.1016/j.drudis.2022.103458.
17 L. Ceze, J. Nivala and K. Strauss, *Nat. Rev. Genet.*, 2019, **20**, 456-466.
18 (a) H. Hori, *Genes-Basel*, 2023, **14**, doi:10.3390/genes14020382; (b) L.Z. Zhang, Y.G. Zhang, S. Zhang, L. Qiu, Y. Zhang, Y. Zhou, J.H. Han and J. Xie, *Genes-Basel*, 2022, **13**, doi:10.3390/genes13112050.
19 Y.Y. Komal Chillar, Dhananjani N. A. M. Eriyagama, Shiyue Fang, *PeerJ Anal. Chem.*, 2022, doi:10.7717/peerj-achem.7720.