Supporting Information

for

Electrophilic oligodeoxynucleotide synthesis using dM-Dmoc for amino protection

Shahien Shahsavari, Dhananjani N. A. M. Eriyagama, Bhaskar Halami, Vagarshak Begoyan, Marina Tanasova, Jinsen Chen and Shiyue Fang


HPLC profiles, MALDI–TOF MS spectra, UV spectra, and OD$_{260}$ values of ODNs, and NMR spectra of new compounds
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RP HPLC profile of purified trityl-tagged ODN 5'-TTA TCC ACT TCC GTT CTA CT-3' (30a-tr).
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RP HPLC profile of crude trityl-tagged ODN 5'-TTA TCA AAC TTG TAA CCC CT-3' (30b-tr). The peak at 35–39 min corresponds to the trityl-tagged ODN. The peaks after 40 min correspond to branched sequences.

RP HPLC profile of purified trityl-tagged ODN 5'-TTA TCA AAC TTG TAA CCC CT-3' (30b-tr).
RP HPLC profile of de-tritylated ODN 5’-TTA TCA AAC TTG TAA CCC CT-3’ (30b).

RP HPLC profile of pure de-tritylated ODN 5’-TTA TCA AAC TTG TAA CCC CT-3’ (30b).
A typical RP HPLC profile of crude ODN (5'-CTA GAT AAC TCA TAG TAC TT-3') synthesized using 3a–c and 4 under standard conditions using acetic anhydride for capping and without 5'-tagging with hydrophobic groups such as trityl and DMTr groups. The peak between 19 and 21 min corresponds to the ODN. The peaks after 21 min correspond to branched sequences. Because the desired ODN and branched sequences were very close, ODN purification was difficult.

RP HPLC profile of the crude ODN 5'-DMTr-O-TTC CAT CCT AGA AAG CTC AT-3' synthesized using 3a–c and 4 under standard conditions using acetic anhydride for capping. At the end of synthesis, the DMTr group was not removed. Although not always possible, in this case, the DMTr protection survived the cleavage and deprotection conditions involving sodium periodate. The peak in the profile between 43 and 45 min corresponds to the DMTr-tagged ODN. The peaks after 47 min correspond to branched sequences. The branched sequences have longer retention times because they have two or more 5'-ends and thus have two or more DMTr groups.
A typical RP HPLC profile of crude ODN (5'-TBDPS-O-CTA GAT AAC TCA TAG TAC TT-3') synthesized using 3a-c and 4 under standard conditions using acetic anhydride for capping and tagged with a TBDPS group at the 5'-end. The TBDPS, which is the t-Bu(Ph₂)Si-group, was introduced after solid phase synthesis (5'-DMTr group removed) and before cleavage and deprotection by soaking the CPG in 0.1 M t-Bu(Ph₂)SiCl and 0.1 M imidazole in DMF (rt, 12 h). Cleavage and deprotection were then carried out as described in the article. The peak between 41 and 42 min corresponds to the tagged ODN. The peaks after 43 min correspond to branched sequences. The branched sequences have longer retention times because they have two or more 5'-ends and thus have two or more TBDPS groups. The approach separated the desired ODN from the branched sequences very well, but at this stage, we cannot identify a mild condition that is compatible with sensitive modifications on ODNs to remove the TBDPS group after the ODN is purified.
RP HPLC profile of crude trityl-tagged ODN 30c-tr. The peak at 37–40 min corresponds to the trityl-tagged ODN. The peaks after 40 min correspond to branched sequences.

RP HPLC profile of purified trityl-tagged ODN 30c-tr.
RP HPLC profile of de-tritylated ODN 30c.

RP HPLC profile of purified de-tritylated ODN 30c.
RP HPLC profile of crude trityl-tagged ODN 30d-tr. The peak at 37–40 min corresponds to the trityl-tagged ODN. The peaks after 40 min correspond to branched sequences.

RP HPLC profile of purified trityl-tagged ODN 30d-tr.
RP HPLC profile of de-tritylated ODN 30d.

RP HPLC profile of purified de-tritylated ODN 30d.
RP HPLC profile of crude trityl-tagged ODN 30e-tr. The peak at 37–40 min corresponds to the trityl-tagged ODN. The peaks after 40 min correspond to branched sequences.

RP HPLC profile of purified trityl-tagged ODN 30e-tr.
RP HPLC profile of de-tritylated ODN 30e.

RP HPLC profile of purified de-tritylated ODN 30e.
MALDI-TOF MS of trityl-tagged ODN 30a-tr
Calcd for [M-H]\(^{-}\) 6207.1, found 6207.2

Trityl-5'-TTA TCC ACT TCC GTT CTA CT-3' (30a-tr)

MALDI-TOF MS of de-tritylated ODN 30a
Calcd for [M-H]\(^{-}\) 5965.0, found 5964.9

5'-TTA TCC ACT TCC GTT CTA CT-3' (30a)
MALDI-TOF MS of trityl-tagged ODN 30b-tr
Calcd for [M-H]- 6249.1, found 6249.2

Trityl-5'-TTA TCA AAC TTG TAA CCC CT-3' (30b-tr)

MALDI-TOF MS of de-tritylated ODN 30b
Calcd for [M-H]- 6007.0, found 6007.1

5'-TTA TCA AAC TTG TAA CCC CT-3' (30b)
MALDI-TOF MS of trityl-tagged ODN 30c-tr
Calcd for [M-H] \(^{-}\) 6250.2, found 6250.7

MALDI-TOF MS of de-tritylated ODN 30c
Calcd for [M-H] \(^{-}\) 6008.1, found 6008.0
MALDI-TOF MS of trityl-tagged ODN 30d-tr
Calcd for [M-H] 6174.1, found 6174.2

MALDI-TOF MS of de-tritylated ODN 30d
Calcd for [M-H] 5932.0, found 5932.1
MALDI-TOF MS of trityl-tagged ODN 30e-tr
Calcd for [M-H]$^-$ 6253.1, found 6253.1

MALDI-TOF MS of de-tritylated ODN 30e
Calcd for [M-H]$^-$ 6011.0, found 6010.9
UV of ODN 30a.

CPG (4, loading 26 µmol/g, 20 mg) of 0.52 µmol synthesis was divided into 10 portions. One portion was deprotected and cleaved under non-nucleophilic conditions as described in the experimental section. After HPLC purification, the ODN was dissolved in 2 mL water and the above UV spectrum was measured. Thus, the OD\textsubscript{260} of the ODN obtained from the 0.52 µmol synthesis is 2.94 (0.147 × 20), which corresponds to a 3.1% overall yield.

For comparing with standard technology, 30a was synthesized on the same amount of CPG 4 (loading 26 µmol/g, 20 mg) using commercial phosphoramidites under synthesizer manufacturer recommended conditions. The CPG was divided into 10 portions, one portion was deprotected and cleaved with concentrated NH\textsubscript{4}OH (55 °C, 12 h). After HPLC purification, the OD\textsubscript{260} was measured the same way and found to be 8.30 (0.415 × 20), which corresponds to a 8.8% overall yield. As can be seen, even though significant amount branched sequences were observed in the HPLC profile of crude 30a synthesized with the dM-Dmoc technology, the yield of pure target ODN was not significantly lower than that obtained with standard technology. In addition, it is important to note that the standard technology cannot be used to synthesize electrophilic ODNs such as 30c–e.
UV of ODN 30b.

CPG (4, loading 26 µmol/g, 20 mg) of 0.52 µmol synthesis was divided into 10 portions. One portion was deprotected and cleaved under non-nucleophilic conditions as described in the experimental section. After HPLC purification, the ODN was dissolved in 2 mL water and the above UV spectrum was measured. Thus, the OD\textsubscript{260} of the ODN obtained from the 0.52 µmol synthesis is 2.98 (0.149 × 20), which corresponds to a 2.8% overall yield.
UV of ODN 30c.

CPG (4, loading 26 µmol/g, 20 mg) of 0.52 µmol synthesis was divided into 10 portions. One portion was deprotected and cleaved under non-nucleophilic conditions as described in the experimental section. After HPLC purification, the ODN was dissolved in 2 mL water and the above UV spectrum was measured. Thus, the OD260 of the ODN obtained from the 0.52 µmol synthesis is 2.32 (0.116 × 20), which corresponds to a 2.4% overall yield (UV absorption of the unnatural portion of the ODN is not included in the calculation).
UV of ODN 30d.

CPG (4, loading 26 µmol/g, 20 mg) of 0.52 µmol synthesis was divided into 10 portions. One portion was deprotected and cleaved under non-nucleophilic conditions as described in the experimental section. After HPLC purification, the ODN was dissolved in 2 mL water and the above UV spectrum was measured. Thus, the OD_{260} of the ODN obtained from the 0.52 µmol synthesis is 4.68 (0.234 × 20), which corresponds to a 4.6% overall yield (UV absorption of the unnatural portion of the ODN is not included in the calculation).
UV of ODN 30e.

CPG (4, loading 26 µmol/g, 20 mg) of 0.52 µmol synthesis was divided into 10 portions. One portion was deprotected and cleaved under non-nucleophilic conditions as described in the experimental section. After HPLC purification, the ODN was dissolved in 2 mL water and the above UV spectrum was measured. Thus, the OD_{260} of the ODN obtained from the 0.52 µmol synthesis is 6.68 (0.334 × 20), which corresponds to a 7.0% overall yield (UV absorption of the unnatural portion of the ODN is not included in the calculation).
$^{13}$C NMR of 17 in CDCl$_3$, 100 MHz

$^1$H NMR of 18 in CDCl$_3$, 400 MHz
$\text{H NMR of 22 in CDCl}_3, 400$ MHz

$\text{C NMR of 22 in CDCl}_3, 100$ MHz
$^1$H NMR of 3c in CDCl$_3$, 400 MHz

$^{13}$C NMR of 3c in CDCl$_3$, 100 MHz
$^1$H NMR of 28 in CD$_3$OD, 400 MHz

$^{13}$C NMR of 28 in CD$_3$OD, 100 MHz
$^1$H NMR of 29 in CDCl$_3$, 400 MHz

$^{13}$C NMR of 29 in CDCl$_3$, 100 MHz
$^{1}H$ NMR of 26a in CDCl$_3$, 400 MHz

$^{13}C$ NMR of 26a in CDCl$_3$, 100 MHz
$^{31}$P NMR of 26a in CDCl$_3$, 162 MHz