

For research article

Response to Reviewer 2 Comments

1. Summary

Dear Reviewer:

Thank you very much for taking the time to review this manuscript. Please find the detailed responses below and the corresponding revisions/corrections in track changes in the re-submitted files.

2. Questions for General Evaluation	Reviewer's Evaluation	Response and Revisions
Does the introduction provide sufficient background and include all relevant references?	<u>Yes</u> /Can be improved/Must be improved/Not applicable	Thanks for the positive rating.
Are all the cited references relevant to the research?	Yes/Can be improved/Must be improved/Not applicable	N/A
Is the research design appropriate?	<u>Yes</u> /Can be improved/Must be improved/Not applicable	Thanks for the positive rating.
Are the methods adequately described?	<u>Yes</u> /Can be improved/Must be improved/Not applicable	Thanks for the positive rating.
Are the results clearly presented?	<u>Yes</u> /Can be improved/Must be improved/Not applicable	Thanks for the positive rating.
Are the conclusions supported by the results?	<u>Yes</u> /Can be improved/Must be improved/Not applicable	Thanks for the positive rating.

3. Point-by-point response to Comments and Suggestions for Authors

Comments 1: This paper by Chillar et al. reports ultra-mild DNA syntheses for modified oligonucleotides (ONs) with acylated cytosine, adenine, and guanine nucleobases. The authors prepared phosphoramidite monomers bearing N4-methoxycarbonyl-dC, N6-acetyl-dA, N2-acetyl-dG, together with commercially available N4-acetyl-dC to synthesize ONs with acetyl groups. Since these groups are all base-labile, they adopted methyl 1,3-dithian-2-yl-methoxycarbonyl (meDmoc) linker to tether first nucleoside unit to the CPG support, replacing dimethoxytrityl (DMT) group with trityl (Tyr) group for the protection of 5'-OH, which enables two-step cleavage from the support and ultramild deprotection of resulting ONs with non-nucleophilic K_2CO_3 . The authors successfully confirmed that all of the three base-labile acyl-groups on nucleobases are intact in the cleavage and deprotection process, giving slight shouldered HPLC peak but sharp single peaks in capillary electrophoresis and MALDI-TOF/MS. Experimental setup and results look convincing, and the logical flow of the manuscript is reasonable. I recommend acceptance for publication

after the following minor points are addressed.

Response 1: Thanks the reviewer for the accurate summary of the paper.

Comments 2: Term "sensitive": I think "base-labile" is the most standard term to represent the "sensitivity" of protective groups in organic chemistry as well as in nucleic acid chemistry. I recommend replacing at least the one found in the Abstract (line 8) for easier understanding of the concept by readers in the field.

Response 2: Thanks for the suggestion. It is an important one because the change will make the paper more searchable. The suggested change is made. See line 8.

Comments 3: Full spelling of "meDmoc" and "Dmoc" should be presented in the Abstract (lines 11 and 12).

Response 3: Thanks for the comment. Full spelling has been added for the two terms. See 11-13.

Comments 4: Figure 2: the lower part of the "Figure" showing the pathway from compound 3 to 6 is a reaction "Scheme" in fact. I recommend to add "Scheme 1" for the part.

Response 4: This Figure/Scheme along with related sites in the text has been changed. See the Track-Changes-On version of the revised manuscript for the multiple changes.

4. Response to Comments on the Quality of English Language

Point 1: N/A

Response 1: N/A

5. Additional clarifications

We appreciate the reviewer for the constructive comments. They are very helpful for improving the manuscript. Thanks for your service for the scientific community.