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Observation and Elimination of *N*-Acetylation of Oligonucleotides Prepared Using Fast-Deprotecting Phosphoramidites and Ultra-Mild Deprotection

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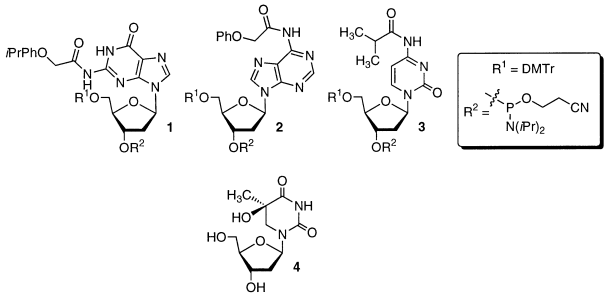
Abstract—Commercially available 'fast-deprotecting' phosphoramidites are useful for synthesizing oligonucleotides containing alkali-sensitive nucleotides. However, *N*-acetylated oligonucleotides were observed during solid-phase synthesis using 'fast-deprotecting' phosphoramidites in conjunction with K_2CO_3/MeOH ('ultra-mild') deprotection. Transamidation was localized at deoxyguanosine, which is protected as its isopropylphenoxyacetyl amide. Substitution of trimethylacetic anhydride for acetic anhydride and appropriate modification of the automated synthesis cycles eliminated this problem.

Oligonucleotides containing labile nucleotides are useful for mechanistic and diagnostic studies.[1–3] The need for oligonucleotides containing these types of molecules provides a driving force for developing solid-phase biopolymer synthesis methods. Significant efforts have focused on developing phosphoramidites containing exocyclic amine protecting groups, which can be deprotected under mild conditions. Amino protecting groups that can be cleaved using palladium, fluoride, or light have been reported.[4–6] A larger variety of nitrogen protecting groups that are cleaved rapidly under protic conditions (concentrated aqueous ammonia, or K_2CO_3/MeOH) or even aprotic alkaline conditions (e.g., DBU) have also been developed.[2a,7–11] Recently, we reported on a family of exocyclic amine protecting groups that are removed upon treatment with triethylamine.[3]



5'-d(GTC ACG TGC TGC ATA CGA CGT GCT GAG CCT)
5

Commercially available 'fast-deprotecting' phosphoramidites (e.g., **1**–**3**) have been used successfully to incorporate alkali-labile nucleotides (e.g., **4**) at defined sites in chemically synthesized oligonucleotides.[2,8,9] Oligonucleotides containing **4** were deprotected and cleaved from the solid-phase support using anhydrous K_2CO_3 (0.05 M)/MeOH ('ultra-mild' deprotection) and found to be homogeneous by ESI–MS. Recently, we had occasion to prepare other oligonucleotides using this synthesis/deprotection approach. To our surprise, products (e.g., **5**) that appeared to be homogeneous by denaturing gel electrophoresis were shown by ESI–MS to consist of several products containing varying numbers (0–4) of acetate groups, as evidenced by ions possessing m/z ratios greater than that expected for **5** by multiples of ~42 amu (Fig. 1). The acetate groups were cleaved upon overnight treatment with K_2CO_3 or concentrated aqueous ammonia (data not shown). However, these conditions are incompatible with sensitive nucleotides, such as **4**.

Subsequent investigations confirmed that impurities in the phosphoramidites were not the source of the acetylated oligonucleotides. Synthesis and deprotection (K_2CO_3/MeOH) of dA_{20} and dC_{20} yielded oligonucleotides that exhibited a single ion in the ESI–MS with the expected m/z ratio (data not shown). Based upon these observations we speculated that the deoxyguanosine nucleotides in **5** were selectively acetylated during capping, and that *N*-acetylation was occurring during the capping procedure. One explanation for this occurrence was that the isopropylphenoxyacetyl protecting group (*i*Pr-Pac) could be partially cleaved during one or more

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of the steps during the synthesis cycle (Scheme 1). The free amine would then be acetylated. Alternatively, we considered that the protected amine could be further acylated by the *N*-methylimidazole (NMI) activated acylium ion. The *i*Pr-Pac group of the bis-amide would then be preferentially cleaved. Of the two transamidation mechanisms (Scheme 1), the latter was considered to be more likely. Furthermore, this process had previously been proposed during the development of *t*-butylphenoxyacetyl protecting groups.[9] Consequently, we reasoned that use of a more hindered capping agent would eliminate the undesired transamidation process.

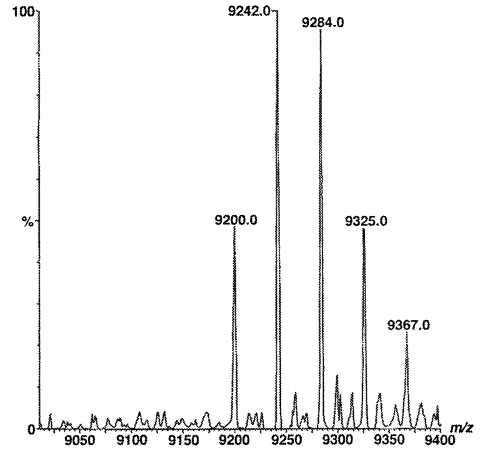
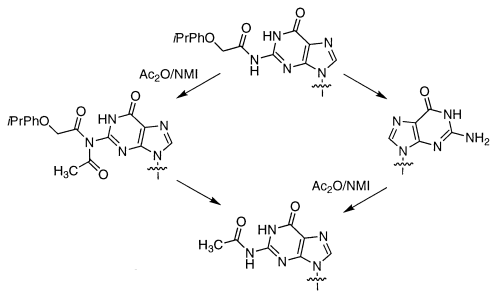


Figure 1. ESI–MS of **5** prepared using acetic anhydride as capping agent and K_2CO_3/MeOH during deprotection. The calculated *m*/*z* of **5** = 9199.0.



Scheme 1.

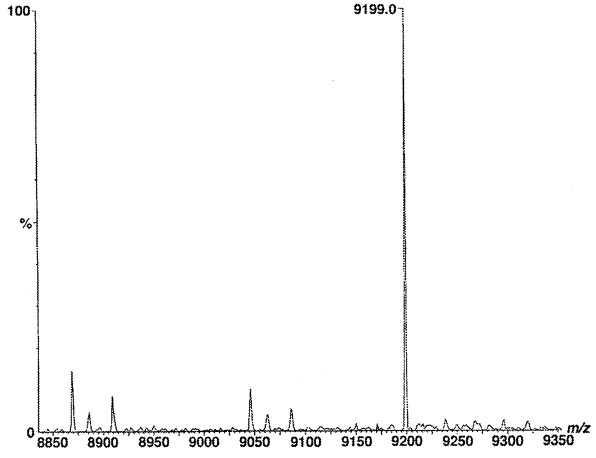


Figure 2. ESI–MS of **5** prepared using trimethylacetic anhydride as capping agent and K_2CO_3/MeOH during deprotection. The calculated *m*/*z* of **5** = 9199.0.

Substitution of trimethylacetic anhydride (10% by volume, 0.49 M) for acetic anhydride in the capping solution containing 2,6-lutidine in THF, and utilization in the attempted synthesis of **5** using unaltered DNA synthesis cycles yielded oligonucleotide product containing no extraneous acyl groups. However, the desired product was difficult to separate from the failure sequences, and we surmised that the more hindered anhydride was less efficient at capping the sequences that failed to couple. Extension of the capping time to 20 s from 5 s eliminated this problem. Following deprotection with K_2CO_3, **5** (Fig. 2) was obtained in yields comparable to that obtained using 'fast-deprotecting' phosphoramidites and concentrated aqueous ammonia during deprotection.

In conclusion, we recommend that trimethylacetic anhydride be substituted for acetic anhydride in the capping step of solid-phase oligonucleotide synthesis when 'fast-deprotecting' phosphoramidites are used in conjunction with 'ultra-mild' deprotection (K_2CO_3/MeOH).[11]

Recommended Solid-Phase Oligonucleotide Synthesis Procedure for Using 'Fast-Deprotecting' Phosphoramidites and K_2CO_3/MeOH Deprotection

When using an ABI 392/394 oligonucleotide synthesizer and phosphoramidites at 0.05 M change the wait time following transfer of Cap A and Cap B solution to 20 s. Replace Cap A solution with one containing a mixture of THF/2,6-lutidine/trimethylacetic anhydride (8:1:1 by volume). Oligonucleotides were treated with 0.05 M K_2CO_3 in anhydrous methanol for 2 h at 25 °C. K_2CO_3 was dried in a drying pistol under vacuum in the presence of P_2O_5. The supernatant was treated with 2 molar equiv of acetic acid, and then concentrated to dryness prior to purification by denaturing gel electrophoresis.

Acknowledgements

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References

1. Beaucage, S. L.; Iyer, R. P. *Tetrahedron* **1993**, *49*, 6123.
2. (a) Sambandam, A.; Greenberg, M. M. *Nucleic Acids Res.* **1999**, *27*, 3597. (b) Iwai, S. *Angew. Chem. Int. Ed.* **2000**, *39*,

3874. (c) Iwai, S.; Shimizu, M.; Kamiya, H.; Ohtsuka, E. *J. Am. Chem. Soc*. **1996**, *118*, 7642.
3. Chen, T. C.; Fu, J.; Greenberg, M. M. *Org. Lett*. **2000**, *2*, 3691.
4. Hayakawa, Y.; Wakabayashi, S.; Kato, H.; Noyori, R. *J. Am. Chem. Soc*. **1990**, *112*, 1691.
5. Stutz, A.; Hobartner, C.; Pitsch, S. *Helv. Chim. Acta* **2000**, *83*, 2477.
6. Stutz, A.; Pitsch, S. *Synlett* **1999**, 930.
7. Iyer, R. P.; Beaucage, S. I. In *Comprehensive Natural Products Chemistry*; Kool, E. T., Ed.; Elsevier: Amsterdam, 1999; Vol. 7, pp 105–152.
8. (a) Schulhof, J. C.; Molko, D.; Teoule, R. *Tetrahedron Lett*. **1987**, *28*, 51. (b) Vu, H.; McCollum, C.; Jacobson, K.; Theisen, P.; Vinayak, R.; Spiess, E.; Andrus, A. *Tetrahedron Lett*. **1990**, *31*, 7269.
9. Sinha, N. D.; Davis, P.; Usman, N.; Pérez, J.; Hodge, R.; Kremsky, J.; Casale, R. *Biochimie* **1993**, *75*, 13.
10. (a) Himmelsbach, F.; Schulz, B. S.; Trichtinger, T.; Charubala, R.; Pfleiderer, W. *Tetrahedron* **1984**, *40*, 59. (b) Stengele, K.-P.; Pfleiderer, W. *Tetrahedron Lett*. **1990**, *31*, 2549.
11. 'Ultra-mild' deprotection of 'fast-deprotecting' phosphoramidites is also described in the Glen Research 2000 catalog, p 13.